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ECOLOGICAL ASPECTS OF FLOWER EVOLUTION. I. PHYLETIC EVOLUTION [1]

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Ein /phylogenetischer/ Erklärungsversuch muss auch ökologisch verständlich sein.
R. v. Wettstein.

A century has elapsed since Darwin in the "Origin of Species" (1859) put the flower-insect relation into the framework of natural selection. Darwin revived the ideas of Sprengel and Knight and placed them in a new frame. He noted that a trifling difference in the flower of red clover or in the tongue length of its bee visitors might be decisive for the reproduction of the plant. If bumblebees should disappear, the red clover might be saved by a shorter tube. In 1857 and 1858 Darwin published observations on "The agency of bees in papilionaceous flowers," proving the advantage of cross-pollination. The book on "The Various Contrivances by which Orchids are Fertilised by Insects" was published in 1862, and "The Effect of Cross- and Self-fertilization in the Vegetable Kingdom" in 1876. In these and the following years Darwin inspired the old masters, the two Müllers, Hildebrand, and even Delpino, though the latter botanist took an independent position, preferring a more philosophical approach.

Towards the end of the 19th century the building of floral biology seemed completed, as concluded in Knuth-Loew's "Blütenbiologie" (1898–1904). Various authors, however, described more and more instances where self-pollination seemed biologically sound and where flower organs were not adapted for cross-pollination. Many authors then started to doubt the importance of cross-pollination, of specific pollinators and of natural selection.

One can use the term "adaptive" to describe a functional relation in a higher unity without regard to a selective system which may have brought it about. So we shall have to distinguish first the recognition of the unity between a certain flower and a certain pollinator and secondly, the explanation of its origin.

Anti-selectionists such as Goebel, Troll, and Melin claimed that the hunt for adaptations has led to baseless assumptions. In their negativism, however, they came,

[1] The list of literature cited appears at the end of the second article in this series scheduled for the March 1961 issue of EVOLUTION (vol. 15, no. 1).

as we shall see, to a denial of adaptation in the flower in every respect. We may recall that after 1912 all flower colors were temporarily branded as useless because bees were thought to be color-blind. For a collection of "scientific, anti-teleological" ideas from those days one might consult Velenovsky's "Vergleichende Morphologie" (pp. 1079–1087). One feels in his final lines against the concept of the flower as *geistloser Kopulationsmechanismus* some trace of Goethe's aesthetic aversion to sexuality in the flower. The general attention of botanists, moreover, shifted to measurable bonds between plants and climatic factors. The biotic sphere appeared negligible in temperate regions, where small climatic differences seemed to determine a multiplicity of forms.

Flower evolution can be considered from different viewpoints. Although we are taking the ecological approach in this paper, I have to take a position in regard to other viewpoints when they overlap with our present field. Let us consider first some purely morphological concepts of the flower.

Of course ecology should recognize given morphological elements and possibilities. In his "Organography" and in "Die Entfaltungsbewegungen" Goebel (1915–1920) tried to consider the variations of flower organs as products of a primary, innate pluriformity. The environment just uses these diverse forms. Function is *"Ausnützung"* (utilization). He was strongly opposed to selection as a creative force, and to the term adaptation, and tried to reduce apparent adaptations to repetition of unfolding movements and the like. Necessary as his attitude was against uncritical amateurism, his mighty work has by its one-sidedness also exerted a negativistic influence on minor gods.

Some anti-Darwinists prefer a more or less metaphysical autonomous morphology with the accent on orthogenesis. Cronquist (1951) advocated orthogenesis on a genetically justified basis. He found parallel trends of development in different groups of Compositae.

Recently Good (1956), using such data, published a book "Features of Evolution in the Flowering Plants." This is very one-sided, neglecting genetics completely, as well as floral ecology and all bonds between flower and environment. Good considers evolution as motiveless, automatic autogenesis. This brings, as he says, an element of repetition into floral forms, a kaleidoscopic elaboration.

I think, however, we may consider the repetition of the form of *Fuchsia* in a Composite like *Mutisia* as an ecological convergence due to ornithophily. In a critical review of Good's book I already suggested (van der Pijl, 1958) that many of the mentioned repetitive morphologisms, that even the characters of Monocotyledons show correlations with each other and with their original life habits. Such "repetitions" may be simply the same answer to the same challenge.

Mangenot (1951) also described apparently autonomous repetitions on a grand scale, evolutionary cycles of envelopment in seed and fruit.

In morphological studies the term "protection" is often used for such processes without indicating against what.

The foregoing ideas lead to the idealistic morphology of workers like Troll (1928), who have discarded the functional aspects found in Goebel's work. Troll believes in independent ideas, *"Gestalten,"* which govern the given organization schemes, make a flower again out of an inflorescence. They are supra-material factors and need no explanation. It is remarkable that Troll recognized convergent adaptation in animals, e.g. whales becoming fish-like, but not in plants.

In my opinion the splendid work of Vogel (1954) suffers from the influences of his teacher Troll, including Goethean reminiscences of "esthetic values" (cf. Grant, 1958). His observations underline the static existence of flower-classes

according to their visitors following Delpino. Being a good observer he does, of course, recognize the ecological significance of their characters, but calls them *"Stil"* (style). Flowers and insects are, in his opinion, not adapted because their organs fit mutually after independent development, but rather their organs exist by the regulation of a holistic plan. Adaptation without selection!

Though Vogel accepts phylogeny he is led to his concept because he is struck by the remarkable co-adaptation of the flower parts, their necessary, mutual co-ordination, which makes it difficult to escape the impression of an existing plan behind the refined and bewildering structures of some Asclepiadaceae and Orchidaceae. He obviously shares for flowers the well-known judgment of Bergson for the animal eye, that independent changes of parts would necessarily disturb the general plan, and he also shares another classical argument, that small initial changes can have had no selective value.

I will only ask some questions: (1) How are adaptations of flowers to abiotic, climatic factors to be considered? (Note: Vogel and Troll neglect the reversion to anemophily.) (2) How do we conceive of *Gestalt* or *Stil* of flowers in the beetle-era? (3) Is there also just *Stil* behind the vegetative convergence in *Euphorbia-Cereus-Stapelia?* (4) What is the *Stil* of neutral and transitional forms? (5) Do characters which exclude certain animals belong to the *Stil?*

The flower can also be considered from a purely physiological standpoint. Sometimes a process or structure in a flower may be just a physiological necessity. Though the flower is, physiologically, so to speak, a dissipation the processes of which are diverted for external use, it remains, of course, susceptible to physiological laws. A small dislocation in physiology during initiation can have profound consequences. But physiological explanations do not do away with function.

Cammerloher (1925) carried out experiments on the direct cause of the resupination of *Canavalia*-flowers (to be discussed under *Xylocopa*-flowers). When finding the physiological induction, he concluded that therefore there was no question of significance or of adaptation for specific pollinators. Of course this reasoning, influenced by Goebel, is ecologically incorrect (cf. van der Pijl, 1954, p. 418).

Nelsson (1954) extended this reasoning in a book on the "Laws Governing Change of Form in the Flower." He thinks that a physiological approach is sufficient and final. As Nelsson used a simplistic kind of physiology—mainly better or less nutrition of flower parts—and ignored floral ecology, he reached queer conclusions. I cite some. "The likeness of *Ophrys* to a bee is useless." "Heteranthery and heterostyly are functionless, physiological consequences of position." "The simple form of *Calycanthus*-flowers is a mutual influence of vegetative and reproductive plans." (One asks in regard to such propositions: Why in some flowers only?). He paints a pistil with shellac, leaving the nectar-guide intact. When this flower is not visited, he considers this as proof that the nectar-guide is useless, etc., etc.

In the second quarter of the 20th century a renewal of the ecological approach to flowers set in. Its causes seem manifold: firstly, the new approach of population genetics, secondly, the new evidence from tropical floral biology, further, the evidence from paleontology, and, finally, the experimental approach to the flower-insect relationship.

Early ideas on the function of entomophilous traits in flowers were mere suppositions, open to the severe criticism of Goebel and allies. The pioneer in the field of experimental research of these relations was Knoll (1921–1926). Some of his brilliant early results had (and perhaps others are) to be corrected. Later on the problem passed entirely into the hands of zoological physiologists.

Modern experimental work confirmed the real importance of colors, structures and odors, refined insight into their value for respectively distant and near "attraction," for orientation and tongue-reactions of bees and butterflies. It confirmed, in contrast to lower pollinators, their preference for special forms, for zygomorphous symmetry, for three-dimensional configurations (*Tiefenwirkung*) often acting as a complex. A dissected contour and broken pattern are important for the reaction of alighting (Manning, 1956a). Ray florets thus give more than a mere enlargement of surface. The sensitivity of many birds for the contrast red-green was confirmed. For carrion-fly flowers the utility of a chequered panther-design was also proved (Steiner, 1948). Further research will have to decide whether the important factor of ultra-violet reflection, by which two white flowers may be ecologically different, plays a role in differentiation. The visual honey-guides may be amplified by odor-guides (*Duftmale* of Lex, 1954).

Though this whole matter should be discussed elsewhere and I am foreign in the field, I mention it here to have the main points in this context and for use in the following parts. It would be interesting to know how much of Goebel's criticism has been wiped away. Werth (1956) who is strongly anti-selectionistic, thought he saw a contrast between Darwinism and experimental floral ecology. The reverse seems true. The experiments, though not forming a direct proof of the action of selection in past evolution, do provide a basis for it.

During the last few years many books on general floral ecology have appeared. To cite only those in world-languages: The popular one by Knoll (1956), and those by Vogel (1954) and the zoologist Kugler (1955). The book by Werth (1956) (cf. the review by Grant, 1958) belongs in regard to many of its data, its one-sided references and general attitude to the first quarter of the century. The floral biology by James and Clapham (1935) contains only an elaboration of some European flower mechanisms. Jaeger (1957) published an extensive review of 255 references, which treats only entomophily, and this rather statically. Of older books I mention a short popular one by Cammerloher (1931) never completed, giving a general introduction with a somewhat tropical orientation. Kirchner (1911) gave a more detailed review of pollination and flower classes in Europe.

In the following pages we shall attempt to state a modern viewpoint on the origin, differentiation, and function of the flower. Special attention will be paid to the ecological side of flower evolution. This evolutionary problem has usually been discussed from the classical standpoint of temperate floral ecology. In the present review the contributions of the newer tropical floral ecology will be stressed.

The Genetical Significance of the Flower

The continual mixing of germ-plasms, already stressed by Darwin, H. Müller and Weismann, has been recognized more recently by geneticists as an important evolutionary factor behind sexual reproduction. Gene-ecology, biosystematics and pollination-dynamics have become important branches of biology.

Self-pollination was proved on a new basis to be a danger for progress in evolution. It is a blind alley leading to rapid speciation, but sacrificing the future for mere survival in unfavorable regions, or near the limit of species or genus (cf. Grant, 1954; Stebbins, 1957). Docters van Leeuwen (1933) found a large part of Javanese mountain plants to be autogamous. Hagerup (1932, 1951) described a new instance of this change in northern regions and in the Sahara. The fact that self-fertility is often a consequence of inactivation of sterility genes demonstrates its secondary or derived character.

It has been assumed of old since Burck (also van der Pijl, 1953) that in tropical Annonaceae the curious shedding of

stamens and the closed condition lead to self-fertilization. The results of Wester (1910) show that selfing often does not occur owing to protogyny. Perhaps the closure brings about trapping of beetles, and the shedding of stamens and petals dusts them with pollen and liberates them. Periasamy (1954) demonstrated this for *Polyalthia,* also showed that *Cananga odorata* even has a peculiar mechanism for the prevention of self-fertilization in the dropping of the stigma heads shortly after the dehiscence and shedding of the stamens. Winkler (1906) reported the same mechanism for *Monodora.* He also found that self-fertilization in *Uvaria winkleri* is prevented by protogyny. For other Annonaceae he admitted autogamy.

Whereas Werth (1955) denied the general effect of pollinators on speciation, the Californian school of Stebbins-Grant-Straw offered much proof to confirm it. The combination of floral ecology with cytology and population genetics of this school (cf. Straw, 1956) is an example of modern taxonomic research.

Grant has published many data in papers on Polemoniaceae, soon to be edited as Vol. II of his book on the family. In 1952 he already described an instructive case of ecological separation of two sympatric species by genetic factors which lead to pollination by hummingbirds and hawkmoths respectively. He described how an occasional intermediate hybrid was pollinated by both. The two pollinators were again agents for the splitting of the descendants in the two directions.

Grant has also studied exceptional species adapted to two pollinators, e.g. with a flower with a long tube and much nectar visited by both hawkmoths and hummingbirds. There is evidence that such a species may split by different local selection into forms. One develops a pronounced odor and lighter color (in a region where hawkmoths prevail) and the other form, in a region where hummingbirds are prevalent, loses all scent and becomes red. Accurate biosystematic research may reveal the dynamics of this field, which has so often been treated statically.

The importance of flower constancy of bees as a barrier against crossing in a mixture of species or subspecies growing together was experimentally proved by Mather (1947) and Grant (1948).

Whereas the genetic effect of dichogamy has been belittled as giving just geitonogamy, some tropical cases show a stronger effect because whole branches or plants may be in either the male or the female phase. Instances are the lychee (Khan, 1929) and the avocado (Stout, 1926).

These few remarks on the subject may suffice to give a prelude and background to the main theme. They may also serve to justify the extrapolation from micro-evolution into macro-evolution.

The Origin of the Flower from an Ecological Viewpoint

1. General

There were insurmountable difficulties in phylogenetic considerations as long as we approached the flower from viewpoints born in temperate countries, such as that bees and butterflies are fundamentally the normal pollinators, that nectaries are the fundamental attraction, that specialized herbs in cold climates are the normal plants, or that the anemophilous Amentiferae are starting points of evolution. I shall tackle the latter aspect first.

Robertson (1904) and Bessey (1897) early refuted the popular idea that wind pollination is primitive in the angiosperms. Robertson could hardly imagine any condition under which it would be advantageous for the ovules to become enclosed in a carpel on the supposition that the original angiosperm was anemophilous.

Later others (e.g. Schwarz, 1955) remarked that the flower as such has no basic traits of anemophily. It follows, moreover, from floral-ecological data on tropical oaks and chestnuts that the tem-

perate Amentiferae have their anemophily as a derived condition in poor climates. The essentially tropical genera *Quercus* and *Castanea* often have whitish, entomophilous flowers with nectar secretion and a distinct caprylic smell, with remnants of a bisexual condition and the flowers placed in erect, stiff catkins (cf. Corner, 1940). Regular insect visits have been found by Ridley (in Rendle, 1925, p. 33) who considered erect catkins as entomophilous. Tropical *Castanea* species and even the California *Castanopsis* spread a strong smell of sperma during anthesis. Porsch (1950) showed that even the temperate *Castanea sativa* is still mainly entomophilous and is visited by archaic visitors such as beetles and flies, though it is on the way to anemophily.

Pendulous catkins seem secondary transformations of erect, cylindric, cantharophilous inflorescences.

Though he recognizes that the reduction in seed number of Amentiferae is a derived, anemophilous character and that some Himalayan Populus species are bisexual, Zimmermann (1959) maintains that Amentiferae, their unisexuality and their anemophily are basic in the Angiospermae (pp. 500, 503, 538).

I hope to show later that the more recent tendency to consider the Polycarpicae sens. lat. as near to the basal angiosperms is justified for flower-ecological as well as morphological reasons. The basic transition from anemophily to insect pollination must have happened before the origin of the angiosperms. Hermann Müller (1877) already gave us the ecological background for this switch-over in a paper which is still valuable. Non-gregarious flowering plants growing in a mixed vegetation are pollinated more efficiently by insects than by wind.

Some botanists in looking for a demonstrable ecological connection of angiospermous flowers with the gymnosperms have pointed to the Gnetaceae as a link. In *Gnetum, Welwitschia,* and *Ephedra* entomophily has been demonstrated. Insects suck the nectar exuded by the ovule and designated as a transformation of the pollination-drop of older anemophilous gymnosperms. I leave aside the question whether this is right and also the morphological arguments against the Gnetaceae as transition forms. For us it is important that Porsch (1916) pointed out that such a change to attract visitors must have been a blind alley. As soon as the ovules became angiovulate their attractive power became nil. There are no indications that ovular secretions have generally remained accessible through an open stylar channel (cf. *Gnetum*) and it would require mystical "transference of function" to consider stigmatic excretion as an early compensation for this loss.

If we go back to the Cycadaceae and Benettittales we find a better connection. In *Macrozamia Reidlei,* Baird (1938, 1939) found pollinating beetles and Hemiptera on the male and female cones. In *Encephalartos* Rattray (1913) observed pollen-eating beetles as pollinators. On the American *Zamia integrifolia* pollen-eating beetles have been collected (Wester, 1910) and I found them on male *Cycas* cones.

We meet here two elements that deserve a further analysis in their relation to the origin of the angiospermous flower, namely beetles and pollen.

2. *Beetles as the First Pollinators*

Paleontology tells us that there were no well-developed Hymenoptera and Lepidoptera fit to act as large-scale pollinators in the Lower Cretaceous or Upper Jurassic, when the first angiosperms lived. The Coleoptera were already abundant and well differentiated and the Diptera developed during these periods. In the preceding Triassic period insects may already have acquired the habit of spore-eating on the cycads. It is clear that beetles and perhaps some flies stood at the cradle of the flower, as Grant (1950a) has emphasized.

Darwin expressed an early opinion in this general direction. H. Müller in Dar-

win's time also recognized the primacy of beetles, but found no examples of true beetle flowers, as he observed in Europe only where the bees are dominant. In the tropics beetles are more numerous and varied. Delpino (1873/1874) did distinguish beetle flowers, designated as "cantharophiles," as, for instance, *Magnolia, Nymphaea,* and *Paeonia,* but this was more or less surmise.

It is to be expected that Kugler will, in later editions of his book, devote to the beetle-flowers more than the one (negative) sentence now to be found on p. 159.

Since Darwin and Müller we have found so much confirmation in primitive, mostly tropical Polycarpicae that these families deserve special research, particularly relict groups like the Degeneriaceae, Himantandraceae, etc., in relict areas such as New Caledonia, Melanesia and New Zealand (cf. Thomson, 1927, and Heine, 1938).

Diels pointed in 1916 to the phylogenetical significance of beetle pollination in Ranales; Porsch (1950, p. 298) did so again. We know now that beetle pollination is important in some Winteraceae and Calycanthaceae (confirmed in the natural habitat by Grant, 1950b) and some tropical and subtropical Nymphaeaceae. In the latter the giant but primitive flowers of *Victoria amazonica* are cantharophilous. The fruity smell is typical. The fact that large beetles were kept imprisoned for one day was known of old. Porsch (1950) determined *Cyclocephala castanea.* Corner (in litt.) found in Manaus large beetles in every flower opened. Though the temperate cases of *Nymphaea* remain uncertain, the California *Nuphar luteum polysepalum* is (according to Grant) pollinated by beetles of the genus *Donacia* and by some flies. *Degeneria* has a cantharophilous smell and form. *Paeonia* (Werth, 1943b) is a certain case and traces have even remained in Ranunculaceae. The old instance of *Eupomatia* was recently confirmed and extended by Hotchkiss (1958). Many new cases were found in the Annonaceae by Wester (1910) and Corner (1940).

Among the primitive Monocotyledons beetle pollination was found in Liliaceae such as *Cordyline* (Thomson, 1927) and *Calochortus* (Grant, 1954), many Cyclanthaceae (Harling, 1958) and Palmae (Wester, 1910).

In the Araceae many instances were found, e.g., *Caladium* and *Philodendron* (Schrottky, 1910), *Amorphophallus* (van der Pijl, 1937), *Typhonium* (Cleghorn, 1914; van der Pijl, 1953), *Sauromatum* and *Dracunculus* (Hatch and Meeuse, 1960; Schmucker, 1930), *Xanthosoma* (Porsch, 1937). Sometimes the beetles are dung- or carrion-insects attracted by an aminoid smell; sometimes insects from fermenting fruits, attracted by a fruity smell. At times beetles and flies are found together, especially in *Arum* (Knoll, 1926).

As we shall see later on, when treating the beetle-flowers as a class, many traces have been found in higher families, even the Compositae, which originated in the beetle-era (Grant, 1950, p. 195). The Amentiferae must already have switched over to anemophily in this era.

Other primitive insects may have assisted and persisted in pollinating flowers. Thrips were pointed to by Hagerup (1954) and Porsch (1957).

The beetles and the flowers differentiated together in or before the Cretaceous. In the first known Angiospermae we already find epigyny, syncarpy, and sympetaly. These so-called organizational characters could seem neutral and non-ecological as long as we considered only bees and butterflies as normal pollinators.

Angiospermy itself also has often been considered as non-ecological. Whitehouse (1950) suggested an explanation for it as the intervention of a stigma acting as a sieve against "unwanted" pollen, thus promoting cross-pollination. We may extend this view when we remember that in the Cycadaceae pollen has a double

effect, first a non-specific one, giving swelling of the seed-coat and leading to "false" seeds with foreign pollen (cf. De Silva and Tambiah, 1952), and secondly, a specific one, leading to fertilization. The stigma in angiosperms may have excluded the non-specific effect.

Perhaps angiospermy was also necessary, as Grant (1950a) suggested, as a protection of the ovules against gnawing beetle-pollinators with dangerous side-effects. Epigyny then is a further protection against rude visitors and also a convergence. In this light, epigyny, like sympetaly, is to be considered not only as a basic morphological character but also as an adaptive convergence. There may also have been more than one way to angiospermy. Grant (1953) thinks that this process of adaptive radiation led to the foundation of most of the modern flowering plant families.

The term "angiospermy" taken literally is less fitting than "angiovulaty," as the process of covering seems to be directed by the needs of the ovules only. The fate of the seeds stands outside the issue. In a series of articles (cf. van der Pijl, 1955a) I stressed that the flower-ecological necessities even tended to outweigh the fate of the seeds. The ancient mode of seed dispersal, involving a fleshy sarcotesta eaten by herbivorous reptiles, was easily disturbed when the seeds remained covered and the carpels were not burst by the growing seeds. This conflict between two ecological processes may be partly the reason that the first angiosperms could not maintain themselves until secondary opening mechanisms and fleshy outer parts re-established the possibility of animal dispersal.

3. The Means of Attraction

Nectaries are regarded nowadays as largely recent improvements for the attraction of animals. I shall discuss the obviously primary means of attraction, which may have contributed to the early diversification of flowers.

(a) Pollen

Darwin (1876, p. 402) stated that pollen was aboriginally the sole attraction to insects. Indeed the spores of ferns, cycads, and Bennettittales must have been discovered as food. Beetles still take pollen everywhere, even on anemophiles (Porsch, 1956).

Recently Porsch (1950, 1954) stressed the primacy of pollen anew against prevalent present opinion. He found many cases where the pollen itself emits a smell, the oldest flower scent. This still occurs in the cycads, *Castanea, Eupomatia* and other beetle-flowers, and in higher pollen-flowers and in some wind-flowers. As we shall see, the zoophilous dispersal utilizing a definite odor has also developed in some mosses and fungi.

Pollen is suited to the gnawing mouth-parts of primitive beetles, flies, Hymenoptera, and even the oldest existing Lepidoptera, the Micropterygidae, still found as pollen-eaters on Ranales like *Caltha* and *Ranunculus*. There remains in later, secondary, pollen-flowers some correlation between pollen as main food and lower pollinators.

The presentation of pollen as a loose material is consistent with its double function as food to be devoured and as material for pollination. Though differentiation of the two functions may have started early (see below), we find separate food-anthers in the higher families only. Pollen mixed with raphids may have acquired protection against eating (Pohl, 1941).

In the orchids, where flowers with an all-or-none precision have developed, the pollen is taken out of the food-sphere. Sometimes, however, the orchids provide imitation pollen for food. Beck (1914) described a case of this phenomenon for which he suggested a return to pollen-eating beetles.

It is seldom realized that the protection of pollen against disastrous consumption by ants is a most important necessity, especially in the tropics. I have pointed

out characters in flowers that may be explained as ant-deterrents (van der Pijl, 1955).

(b) Food Tissues

Solid masses of edible tissue near the sporangia may have been an old means of attraction for semi-dystrophic visitors. In some Ranales, perhaps also in the "carpel-appendages" of *Victoria,* they are still recognizable as transformed stamens—an early separation of the two pollen-functions mentioned above. Though over and again food bodies have developed secondarily in higher flowers, there is still some correlation between food-tissues and beetles, also between food-tissues and lower flowers. Many Polycarpicae use them, e.g. *Eupomatia, Calycanthus, Himantandra, Victoria,* perhaps *Nelumbo,* also the curious lantern type of beetle-flowers of *Hydnora,* a derived, parasitic ranalian (cf. Marloth, 1913). Among cantharophilous Araceae I demonstrated them in *Amorphophallus variabilis* and *Typhonium trilobatum* (van der Pijl, 1937, 1953). Orchids, in their infinite resourcefulness, discovered them again. In the liliaceous *Calochortus* flowers Grant (oral comm.) also found food-bodies for beetles.

(c) Deceit

We may suppose that originally visits of insects were casual. The old argument that flowers without proper flower-insects are impossible is not valid. Many of the present day beetle-flowers and fly-flowers still attract visitors by acting on their instincts without offering a reward, and catch them in a trap. The cup-shaped *Magnolia*-type of flower, like the Benettittalian stand of sporophylls, with its enveloping phyllomes have acted primarily as a trap, not just as an optical attraction. Of course this means irregular pollination, dependent on chance situations.

In *Victoria* such a flower, as the apex of all beetle-flowers, though showy and offering food, is a perfect trap with mobile arresting staminodes, just as in *Eupomatia* and in many *Nymphaea* spp. (Schmucker, 1932). The early appearance of sympetaly (in some Annonaceae and *Aristolochia*) and of epigyny or perigyny (in *Victoria* and *Calycanthus*) in primitive flowers may be early ecologically-determined trends which do not justify the taxonomic displacements associated with such differences elsewhere in the system of angiosperms.

I think that the arrangement of trap-flowers as one class is justified only in higher flowers (Asclepiadaceae and Orchidaceae) that were secondarily transformed into a trap. This class then belongs at the end of a system of flower shapes, as Kugler (1956) places all traps following Werth.

Many Annonaceae imprison beetles. *Calycanthus* also is somewhat of a trap. The traps in Orchidaceae do not imprison visitors and have a different significance as we shall see.

In Araceae the flowers are aggregated and reduced but the enveloping of the inflorescence by a spathe as a trap seems a primary acquisition of the family in its beetle period.

Flat complexes of small flowers, accompanied by an involucrum or not, are also able to collect beetles. The offering of food inside some traps seems not primary, but a collateral or even secondary improvement.

The trapping may be necessary for guiding stupid visitors. Many beetles of lower rank as pollinators have poor power of flight and/or of alighting exactly. Some stimulus may cause them to plunge down abruptly. For some dung- and carrion-beetles this abrupt interruption of flight has been analyzed physiologically. In lower flies the approach is different, but not yet direct and well-aimed as in the Hymenoptera. Deceit and trapping are still used for such insects. After the landing the "attracting" odor may release not only feeding instincts but also instincts for oviposition.

Many present-day beetle- and fly-flowers induce their visitors to deposit eggs. In rare cases the larvae can develop well. In old flowers of *Rafflesia* pupae of flies have been found. I described this for *Alocasia* and *Artocarpus* (van der Pijl, 1933, 1953) as leading to a kind of cyclic symbiosis. Pollinators are thus regularly present and the plant is no longer dependent on some incidental or casual visitor. Hagerup (1956) described another parallel to the *Yucca*-case, *Trollius* and a fly, and *Calluna* and a thrips.

The specificity of the traps is guaranteed by odor and dimensions. The protogyny, still dominant in Ranales, must have been useful in traps.

Werth (1956, p. 110) objected that one cannot imagine how insects could breed deceitful flowers by selection. The plant, however, is favored by it, whereas the insects are not severely harmed so long as deceit remains an exception. The berry-like but undigestible "mimetic" seeds of some Leguminosae (*Abrus*) have always relied on such deceit for their dispersal (cf. van der Pijl, 1955a, 1956a); so do some *Allium* and *Paeonia* species. It is curious (cf. Grant, 1958) that Werth applied his objection to the case of *Parnassia* in which the role of deceit was confirmed already by Kugler in 1951 and not to trap-flowers.

Since the discovery of flower-constancy in bees we have tended to consider colors and odors of flowers as signals for food, not as primary attractants. The direct action on instincts, so evident in flies and sphingids, can, however, not be neglected for bees either (cf. von Frisch below). In connection with the deceitful attraction of male Hymenoptera to some orchids, Kullenberg (1956b) demonstrated a direct action of many odorous compounds on males and females of some Hymenoptera.

(d) Nectar

Nectar secretion may have occurred in the first flowers. Physiologists often object that nectaries are primarily leaf-organs of a physiological nature. Of course they are right. This does not preclude a secondary utilization, however, which Darwin pointed to already in 1876 (p. 401).

Ovular or carpellary secretion causes visits to female parts and edible pollen to male floral parts only. In the days when the primitive flower was considered to be unisexual this result was difficult to explain. The difficulties are not over when we consider *Castanea, Gnetum* and Cycadaceae. Rattray (1913) found beetles eating pollen on the scented male cones of *Encephalartos* and laying eggs on the apparently inodorous female cones. The eggs in the ovules produced new beetles. The two inflorescences thus seem to act on two different instincts of the visitors.

In many Polycarpicae nectar is secreted simply on the face of the carpels and other phyllomes. It is interesting that in some Helobiae, too, the basal surface of the carpels secretes nectar. Porsch (1913) supported the derivation of Monocotyledons via Helobiae from Polycarpicae by deriving the septal nectaries from carpel surfaces enclosed by syncarpy. Daumann (1931) observed primitive nectar-secretion on staminodes and carpellodes in some Helobiae and Araceae. He remarked that the secretion increased with the degree of sterility and that these organs might lead to later "axial" nectaries.

I need not dwell on further complications of nectaries in relation to beetles and other insects with longer mouthparts, but will only mention a unique study of Brown (1938). He pointed out that flower evolution is often limited by the inherent place of nectar secretion. In Malvales the nectaries on the calyx prevent sympetaly. The scarcity of inferior ovaries in Lamiales seems to be partly caused by the position of the nectary.

Fahn (1953) extended these considerations. Such topographical studies should, of course, be supplemented by functional and morphological investigations, as nectaries may be extremely con-

densed structures of the most heterogeneous nature. Some extra-floral nectaries are condensed entire flowers or even inflorescenses (van der Pijl, 1955b).

Extra-floral nectaries have, as we shall see, more recently been drawn again into the sphere of the flower. I pointed out (1955b) that only non-tropical botanists can maintain that they are always ecologically useless. Only neglect of floral ecology allows this thesis to stand for the giant extra-floral nectaries of *Poinsettia* which are so attractive to birds.

The Differentiation of Higher Flowers

The influence of higher insects comes into the picture, along with morphological integration, in the evolution of the more advanced types of flowers. The old suppositions of Darwin and especially H. Müller stand now much reinforced. The first Hymenoptera must have utilized simple, pre-existing flowers. The now existing Sphegidae and Ichneumonidae, already represented in the Cretaceous period, have a knack of finding inconspicuous extra-floral nectaries, and small open flowers in the tropics. Schremmer (1959) showed that a European *Polistes* was also primarily interested in post-floral, inconspicuous nectar. In the Tertiary the higher flower visitors in the Syrphidae, Lepidoptera, Bombineae, and Apoideae became abundant.

It is natural that the influence of higher visitors in natural selection was emphasized by Darwin and the Müllers. New factors of competition in a changing world must have had consequences. Roughly speaking, it is not (as some anti-selectionists think) nonsensical to say that insects shaped the flower anew, causing a new wave of adaptive radiation. The old world was good, but better adapted flowers were possible. Apart from generally better pollination by regular flower-insects like bees, specialization of visitors means better pollination and less wastage of pollen together with faster speciation.

Manning (1956) observed that a bumblebee which strayed onto a *Magnolia* flower was ill at ease; it missed orientation marks and other stimuli, fumbled around, and did not bring about pollination. When our numerous and fast-moving bees are found on an umbelliferous inflorescence together with some beetles, the regular presence of the latter is more significant. If we find *Xylocopa* bees on a hundred kinds of flowers, this does not mean that they do not prefer one to another—as comparative counts reveal. In bird flowers I found striking competition of this kind.

Most botanists now agree that, though each family may have its own trends, higher characters such as deeply hidden nectar, closed corollas with sympetaly and zygomorphy, transformation and reduction of stamens, nectar-guides and intricate pollination mechanisms can be considered as at least initially adaptive and directed by higher pollinators with special senses. The flower became more and more an integrated precision instrument. In the heads of Compositae the same process is on its way. Besides the innate preferences of bees comes a phenomenon of a different nature, viz. the flower constancy (*Blütenstetigkeit*) of bees, replacing searching by fast routine work. It is, however, also important for better and more accurate pollination in mixed vegetation. Grant gave a survey of this field (1950). From the important work of von Frisch on the senses of bees, one item (1947. p. 29, 30) should be cited here: some flower smells do not act by association but by direct stimulation.

The opinion of Leppik (1957) that bees may show preference for certain numerical patterns may contain some confusion between innate preference and flower-constancy of a passing nature. A third flower-insect relation was observed by myself (1954) in tropical carpenter bees (*Xylocopa*), apparently without sign language or pronounced flower constancy. The bees leave in visited flowers an odor signal, perhaps from mandible-

glands, as described by Haas (1952) for other bees. Subsequent visitors of the same bee-species then avoid them for some time. In this way more efficient visiting and pollination of more flowers by scarce bees are obtained. The factor "effective bee-population," (Fryxell, 1957) used in pollination-dynamics, can be influenced by it. *Bombus* also shows signs of using this mechanism, often refusing many individual flowers.

Until now we have considered adaptation from the positive side. There is also a negative side, viz., the exclusion of sub-minimally adapted visitors as already indicated by Darwin (1876, p. 382). Depth is one of the means, though this has also a positive side. The lack of a lip in hummingbird flowers and sphingid flowers is another instance (denied as adaptive by Goebel). We already saw that such antagonistic protective mechanisms (Straw, 1956) are also necessary to balance the dangerous side of legitimate visitors, and we shall find more instances of this.

A stream of papers on the whole subject has appeared from Leppik (1957) in the last years. This author published some interesting schemes. He was somewhat overenthusiastic, stating that "there is no longer any mystery in the evolution of the flower," and points somewhat onesidedly to bees. His schemes are therefore somewhat too rectilinear. The assumed transition of his simplex-type in the Jurassic to the radiatus-type in the beginning of the Cretaceous under the influence of primitive Apidae is perhaps too hasty.

We may now return to some more specific objections against natural selection as a positive force producing directed adaptations. In some localities bees seem to be unable to maintain the balance between protein and carbohydrate or they find pollen which is qualitatively unfit for their larvae (Porsch, 1956, p. 11). For this or some other reason they transfer to pollen of anemophiles (Jaeger, 1954; Porsch, 1955; Heide, 1923; Ducke, 1904, etc.). *Casuarina* and *Zea* are almost the only source of pollen in Queensland (Blake and Rolff, 1939). Melin and others even cited instances of this for hummingbirds. May we now conclude (as has been done) that conspicuous flowers are superfluous?

In a fine study by Manning (1956b) we see that *Bombus,* after some experience on *Cynoglossum* flowers, finally becomes conditioned to the general form of the plant without flowers. May an observer during this phase conclude that flowers are therefore superfluous?

Knoll, an anti-selectionist, concluded from simple early experiments (1926) that flies are not attracted by the purple-brown color of fly-flowers so that this color is not adaptive. Kugler (1956) observed, however, that in the presence of carrion- or dung-odor this color had a clear effect. The Swedish zoologist Melin (1935) launched a sharp attack in the form of a complete book against the adaptive and selective nature of ornithophily. Of the many characters of bird-flowers he found each one lacking in some instances. He concluded, therefore, that each is not necessary and that there is no real ornithophily at all. One of the typical characters is a scarlet color. Indeed it fails many times. It is, however, proved experimentally that hummingbirds can first find their food better if it is indicated by red.

Such arguments consider attraction too simply. There is in flower visitors rarely a conscious searching, looking for beacons. We have to do with animals with instincts, which may be released by stimuli after central coordination. One stimulus may have no effect in itself, but may activate others. The threshold for a certain external factor can be lowered by others. If the threshold is low, only part of the releasing situation will suffice to evoke a response. The release may be just braking the flight, not directing intended visits. Something of this holds true for higher visitors too.

Quite a different argument has been

found in the selective disadvantage bound to eutropy, the narrowing of the circle of visitors, previously described as advantageous. The future of an over-specialized species visited by only one kind of pollinator seems endangered. We see this urge strongly in the Orchids where fruiting is often rare. The most labile and improbable situation is found in the remarkable cases of pollination by pseudocopulation of wasps in the genera *Cryptostylis* and *Ophrys*—again deceit in the sexual sphere. For the extensive literature in this field I refer to Ames (1937). The zoologist Kullenberg (1956a) analyzed the sense-physiological aspect, made a film on the subject and promised a general review. Perhaps *Calochilus campestris* in Australia belongs to the same category (Fordham, 1946) and also *Porphyroglottis maxwelliae* from Malaysia.

To take a well-known case of specialization, mentioned by Darwin, the curious *Angraecum sesquipedale* with its exaggerated spur, seems quite unsafe in its relation with the Sphingid with its exaggerated tongue. It fruits well, however. Darwin predicated in 1862 that such a moth would be found as pollinator. The moth was found in 1903 (cf. Werth, 1943a).

On the other hand the genus *Ficus*, each species with its own gallwasp, is still flourishing. All stenobiotic organisms lead this dangerous life under narrow conditions.

The extreme of one flower species and one kind of visitor, extreme monolecty (monotropy) and eutropy, is rarely reached as completely as in fly-flowers with very specific odors.

Perhaps we may find consolation in high-level philosophy along the line of Vogel. One might say that genus-pluriformity and specialization according to pollinators make the genus stronger against antagonistic influences and more plastic, just as does variability within a species at a lower level.

After all there may be a supra-specific unity on a higher level. Selection, though mechanically understandable, is not antiholistic, it may also be seen as a mechanism obtaining harmony between all life and between life and environment. Perhaps selection is but one of the harmonizing agents in this supra-organism, perhaps there are besides the identifiable hormones of the "organisms" (genes and selection), also invisible and unknown nerves.

Summary

1. Floral ecology received a new impetus and background by Darwin. In the beginning of the 20th century the importance of cross-pollination, selection and adaptation were doubted, but during the second quarter of this century attention increased again.

2. Biosystematic research and population genetics have given a new basis for the evolutionary importance of pollinator-specificity, floral barriers and cross-pollination, as well as for the danger of self-pollination.

3. Tropical ecology also provided support. In Cycadaceae pollination by beetles was and is important. Coniferae are a sideline. The anemophily of Amentiferae proved to be secondary, as tropical relatives are entomophilous. The tropical Polycarpicae are more primitive in respect to pollination.

4. Paleontology shows that beetles must have stood at the cradle of the flower. Clear traces of cantharophily persist in Polycarpicae and some Monocotyledones. Many "neutral and organizational" characters of the first families may have been adaptive in regard to gnawing beetles—as epigyny, syncarpy, staminodes, early sympetaly and even angiospermy itself.

5. The primitive attractions (still important in Polycarpicae) were: (a) pollen with an odor; (b) solid food tissues on sterile organs; (c) diffuse nectar secretion, leading later to nectaries; (d) deceit, trapping of non-adapted beetles by odors. Traps are still used in morpho-

logically primitive groups and have arisen again in Orchids. This provided irregular pollination, dependent on incidental situations.

6. In regard to the differentiation of higher flowers a position is taken on the criticisms of anti-Darwinists like Goebel, Troll, Melin, Good, Nelsson, who denied the adaptive nature of specialized flower structures. Much of their criticism can be disproved, and pure morphology and physiology seem insufficient grounds. The ecological concepts of Darwin, Müller, and followers rightly took into consideration the importance of higher pollinators as regular and more reliable flower-visitors.

7. Researches on the senses of insects provide another new basis for adaptational concepts, by demonstrating the preference of bees for zygomorphy, nectar-guides, dissected contours, deep flowers, and the attractive importance of other characters of flower-classes for special pollinators.

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EVOLUTION OF CELLULAR ANTIGENS IN COLUMBIDAE[1]

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Experiments performed in this laboratory have revealed the complex serological interrelationships of the cellular antigens of a number of species of pigeons and doves (Irwin, 1951; 1953). The studies have shown that red blood cells from the respective species share antigens in common and that they also possess antigens to the exclusion of those found in the cells of one or more other species. It has been demonstrated that the cellular antigens which contrast the different species include several serologically distinct components each of which is determined by a single gene or two or more closely linked genes (Irwin and Cole, 1936; Irwin and Cumley, 1947; Irwin, Cole and Gordon, 1936). The cellular antigens of a particular species may be serologically related to, or identical with, the antigens of one or more other species. The red blood cell antigens ch-1 of *Streptopelia chinensis*, hu-1 of *St. humilis*, or-1 of *St. orientalis*, se-1 of *St. senegalensis* and E of *Columbia guinea* comprise a serologically cross-reactive group (Irwin and Cole, 1940; Stimpfling and Irwin, 1960 and Irwin, unpublished data). In previous papers ch-1 was called d-1, and se-1 was s-2.

Evidence has been obtained for the antithetical relationship of the antigens ch-1, or-1, se-1 and probably hu-1 (Stimpfling and Irwin, 1960). The five serologically related cellular antigens ch-1, hu-1, or-1, se-1 and E have been collectively designated as group-1. A further analysis of the serological and genetic interrelationships within the group-1 system is presented here.

MATERIALS AND METHODS

As a consequence of many generations of successive backcrossing to *risoria* of the hybrids between the respective species and *risoria*, and selected backcross hybrids, the cellular antigens ch-1, hu-1, or-1 and se-1 have been transposed from *chinensis*, *humilis*, *orientalis* and *senegalensis*, respectively, to backcross hybrids whose red blood cells are serologically indistinguishable from the cells of *risoria*, except for the presence of one or another of the corresponding group-1 antigens ch-1, hu-1, or-1 or se-1 (Irwin and Cole, 1936; Irwin and Cumley, 1947; Irwin, unpublished data). The antigen E was transferred to *Columba livia* from *guinea* after successive backcrosses to *livia* of the hybrids between *guinea* and *livia*, and selected backcross hybrids (Irwin, Cole and Gordon, 1936). Blood cells from backcross hybrids possessing antigen E are serologically indistinguishable from the cells of *livia*, except for the presence of E. The backcross birds carrying any one of the antigens of group-1 are descendants of usually a single representative of the respective parental species mated to either *risoria* or *livia* in the production of the species hybrids.

Antisera prepared against the blood cells of the respective backcross hybrids may contain hemagglutinins specific for the cellular antigens of *risoria* and/or *livia* as well as agglutinins against a par-

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ticular group-1 antigen. The species *livia* shares no known antigen specificities with ch-1, hu-1, or-1 or se-1 that it does not also share with *risoria,* therefore, specific reagents for the cellular antigens ch-1, hu-1, or-1 and se-1 were obtained by absorbing their respective antisera with pooled cells from *risoria* and *livia.* This procedure had the added advantage of absorbing from the antisera antibodies which might differentiate individuals within either species. Some anti-E sera, following absorption with cells from *livia,* weakly agglutinated the cells of *risoria,* consequently the anti-E sera were also absorbed with cells from both *risoria* and *livia.*

The available evidence indicates that the antigens se-1 and se-12 are contrasting characters in *senegalensis,* with common as well as differentiating specificities (Stimpfling and Irwin, 1960). Antisera prepared against the cells of se-1 backcross hybrids and the cells of *senegalensis* often contain antibodies reacting with the cells of backcross hybrids possessing se-12. For this reason, se-1 and *senegalensis* antisera were absorbed with cells from se-12 backcross hybrids pooled with cells from *risoria* and *livia* in order to be certain that the serological tests would be limited to the relationship of se-1 to the other antigens of group-1, and not be complicated by antibodies against se-12.

In addition to absorptions of the respective antisera with blood cells from *risoria* and *livia,* the cells of pertinent species and backcross hybrids were also used for absorption. The details are provided elsewhere in this paper.

The serological technics used here have been described in previous publications (Irwin and Cole, 1936; Irwin, Cole and Gordon, 1936; Irwin, 1939). Antisera were obtained following the injection into rabbits of washed blood cells from backcross hybrids possessing one or another of the respective cellular antigens ch-1, hu-1, or-1, se-1 or E. Using the same procedure, other antisera were prepared against cells from each of the species *chinensis, humilis, orientalis, senegalensis* and *guinea.* Washed and packed blood cells were used for absorption. The serological interrelationships were determined by means of the hemagglutination test.

Selected antisera which had been prepared against the cells of backcross hybrids possessing the antigens ch-1, hu-1, or-1, se-1 and E were absorbed at appropriate dilutions with the pooled cells of *risoria* and *livia.* The se-1 antiserum was absorbed, in addition, with the cells of se-12 backcross hybrids. Each of the absorbed fluids was titered against the cells of backcross hybrids having one or another of the antigens ch-1, hu-1, or-1,

TABLE 1. *Absorption analysis of an anti-or-1 serum*

Cells tested	Absorbed with cells of *risoria* and *livia* plus cells of the following											Antigenic specificities
		ch-1	hu-1	or-1	se-1	E	*chinensis*	*orientalis*	*senegalensis*	*quinea*		
risoria	0*	0	0	0	0	0	0	0	0	0	0	
livia	0	0	0	0	0	0	0	0	0	0	0	
ch-1	++**	0	0	0	0	++	0	0	0	0	++	ab
hu-1	++	++	0	0	0	C	++	0	0	0	C	abc
or-1	C	++	++	0	++	C	C	0	0	C	C	abcde
se-1	++	+	++	0	0	++	+	0	0	0	C	abcd
E	++	0	0	0	0	0	0	0	0	0	0	a
chinensis	++	0	0	0	0	++	0	0	0	0	C	ab
humilis	C	C	C	0	C	C	C	0	0	C	C	abcde
orientalis	C	+	+	0	+	C	++	0	0	++	C	abcde
senegalensis	++	±	++	0	0	C	+	0	0	0	C	abcd
quinea	+	0	0	0	0	0	0	0	0	0	0	a
Column No.	1	2	3	4	5	6	7	8	9	10	11	

* "0" indicates the absence of agglutination at a serum dilution of 1:30

** The degree of agglutination is indicated by the following symbols: ± (microscopically detectable), + (weak agglutination, but macroscopically detectable), ++ (strong agglutination), C (complete agglutination).

TABLE 2. *Absorption analysis of an anti-hu-1 serum*

Cells tested	Absorbed with cells of *risoria* and *livia* plus cells of the following											Antigenic specificities
		ch-1	hu-1	or-1	se-1	E	*chinensis*	*humilis*	*orientalis*	*senegalensis*	*quinea*	
risoria	0	0	0	0	0	0	0	0	0	0	0	
livia	0	0	0	0	0	0	0	0	0	0	0	
ch-1	++	0	0	0	0	++	0	0	0	0	+	ab
hu-1	C	++	0	++	++	C	C	0	C	++	C	abfghi
or-1	C	++	0	0	+	C	C	0	0	++	C	abf
se-1	C	++	0	++	0	C	C	0	0	0	C	abg
E	++	0	0	0	0	0	0	0	0	0	0	a
chinensis	C	0	0	0	0	++	0	0	0	0	++	ab
humilis	C	C	0	C	++	C	++	0	++	++	C	abfghi
orientalis	C	C	0	++	+	C	++	0	0	++	C	abfgh
senegalensis	C	++	0	++	0	++	++	0	0	0	C	ab g
quinea	++	0	0	0	0	0	0	0	0	0	0	a

se-1 or E as well as cells from the respective species *chinensis, humilis, orientalis, senegalensis* and *guinea*. The results are given in the first columns of data in tables 1, 2, 3, 4 and 5. Titration of the reagents against the cells of group-1 backcross hybrids and against cells from the respective parental species revealed that following absorption each antiserum contained agglutinins for the homologous cells and for cells of the four heterologous members of group-1. As expected, the group-1 reagents contained antibodies which were also cross-reactive with cells

TABLE 3. *Absorption analysis of an anti-se-1 serum*

Cells tested	Absorbed with cells of *rosiria, livia* and se-12 backcross hybrids plus cells of the following											Antigenic specificities
		ch-1	hu-1	or-1	se-1	E	*chinensis*	*humilis*	*orientalis*	*senegalensis*	*quinea*	
risoria	0	0	0	0	0	0	0	0	0	0	0	
livia	0	0	0	0	0	0	0	0	0	0	0	
se-12	0	0	0	0	0	0	0	0	0	0	0	
ch-1	C	0	C	++	0	C	0	++	++	0	C	abj mn
hu-1	++	0	0	0	0	++	0	0	0	0	++	ab
or-1	C	0	C	0	0	++	0	0	0	0	C	ab m
se-1	C	++	C	C	0	C	++	C	C	0	C	abjklmn
E	++	+	+	+	0	0	+	+	+	0	+	a k
chinensis	C	0	C	++	0	C	0	++	++	0	C	abj mn
humilis	++	+	++	++	0	++	+	0	0	0	++	abj m
orientalis	C	++	C	++	0	C	+	0	0	0	C	abj m
senegalensis	C	++	C	C	0	C	+	++	C	0	C	abjklmn
quinea	++	0	0	0	0	0	0	0	0	0	0	a

TABLE 4. *Absorption analysis of an anti-ch-1 serum*

Cells tested	Absorbed with cells of *risoria* and *livia* plus cells of the following											Antigenic specificities
		ch-1	hu-1	or-1	se-1	E	*chinensis*	*humilis*	*orientalis*	*senegalensis*	*quinea*	
risoria	0	0	0	0	0	0	0	0	0	0	0	
livia	0	0	0	0	0	0	0	0	0	0	0	
ch-1	C	0	C	C	C	C	0	C	C	C	C	ajmnop
hu-1	C	0	0	0	0	0	0	0	0	0	0	a
or-1	C	0	C	0	0	C	0	0	0	0	C	a m
se-1	C	0	C	C	0	C	0	C	C	0	C	ajmn
E	C	0	C	C	C	0	0	C	C	C	0	a o
chinensis	C	0	C	C	++	C	0	C	C	C	C	ajmnop
humilis	C	0	C	C	0	C	0	0	0	0	C	ajm
orientalis	C	0	C	+	0	++	0	0	0	0	++	ajm
senegalensis	C	0	C	C	0	C	0	C	C	0	C	ajmn
quinea	C	0	C	C	++	0	0	++	C	C	0	a o

TABLE 5. *Absorption analysis of an anti-E serum*

Cells tested	Absorbed with cells of *risoria* and *livia* plus cells of the following											Antigenic specificities
		ch-1	hu-1	or-1	se-1	E	*chinensis*	*humilis*	*orientalis*	*senegalensis*	*quinea*	
risoria	0	0	0	0	0	0	0	0	0	0	0	
livia	0	0	0	0	0	0	0	0	0	0	0	
ch-1	C	0	++	++	++	0	0	++	++	++	0	aoqr
hu-1	C	0	0	0	0	0	0	0	0	0	0	a
or-1	C	0	++	0	0	0	0	0	0	0	0	a q
se-1	C	0	C	+	0	0	0	++	++	0	0	a qr
E	C	C	C	C	C	0	C	C	C	C	+	aoqrst
chinensis	C	0	C	++	+	0	0	++	C	++	0	aoqr
humilis	C	0	++	0	0	0	0	0	0	0	0	a q
orientalis	C	0	+	0	0	0	0	0	0	0	0	a q
senegalensis	C	0	++	+	0	0	0	+	++	0	0	a qr
quinea	C	C	C	C	C	0	C	C	C	C	0	aoqrs

from the five parental species. Reciprocally, antisera prepared against cells from each of the parental species *chinensis, humilis, orientalis, senegalensis* and *guinea,* following absorption by the pooled cells of *risoria* and *livia,* were titered against cells from backcross hybrids possessing the antigens ch-1, hu-1, or-1, se-1 and E, respectively. It was observed that the different anti-species sera contained agglutinins for the cellular antigens of each parental species as well as for the antigens of the corresponding derived backcross hybrids. The results indicate that, as would be expected, the group-1 cellular antigens of the respective backcross hybrids and parental species are serologically interrelated.

If the group-1 cellular antigens of the different backcross hybrids and parental species are similar but not identical, it is possible that a particular group-1 antigen may possess a specificity or specificities which distinguishes it from one or more other members of group-1. In order to determine whether or not such differentiating specificities exist each backcross hybrid and species antiserum was absorbed with cells from the respective backcross hybrids and parental species.

A selected antiserum, produced against the cells of backcross hybrids possessing the cellular antigen or-1, was absorbed with the pooled cells of the respective species and backcross hybrids, in addition to cells from *risoria* and *livia.* Each of the absorbed fluids was tested against the cells of two or more representatives of each class of backcross hybrid and each parental species. The results of the agglutination tests with the absorbed reagents are summarized in table 1, columns 2 to 11.

Cells from backcross hybrids possessing the E cellular antigen and cells from *guinea* completely absorbed the agglutinins for themselves and each other but did not absorb the agglutinins for any other species or backcross hybrid (table 1, columns 6 and 11). Cells from each of the species and backcross hybrids listed in table 1 absorbed completely the agglutinins for E and *guinea* from the anti-or-1 serum. It was postulated that the cells of E backcross hybrids and *guinea* possessed at least one specificity shared in common with the or-1 cellular antigen, and with the related antigens of group-1 present on the cells of ch-1, hu-1 and se-1 backcross hybrids and on the cells of the respective parental species. The specificity was designated *a.*

Absorption of the anti-or-1 serum with cells from *chinensis* and ch-1 backcross hybrids removed the agglutinins for themselves and for each other as well as for the cells of *guinea* and E backcross hybrids but not for the cells of any other species or backcross hybrids (table 1, columns 2 and 7. In turn, cells from backcross hybrids carrying hu-1, or-1 and se-1 and cells from *humilis, orientalis* and *senegalensis* absorbed the agglutinins for ch-1 backcross hybrids and *chinensis.*

These results constitute evidence for the existence of a second specificity *b* which is shared by ch-1 and *chinensis* with or-1 as well as with hu-1, se-1 and related antigens on the cells of *humilis, orientalis* and *senegalensis*. The specificities for the cells of *chinensis* and ch-1 backcross hybrids are identified as *a* and *b*.

Cells from hu-1 backcross hybrids absorbed agglutinins for themselves and the cells of backcross hybrids possessing E and ch-1, and also for *guinea* and *chinensis*, but not for the cells of the other backcross hybrids or species (table 1, column 3). Reciprocally, cells from or-1, se-1, *humilis* and *senegalensis* absorbed agglutinins for hu-1. A third specificity *c* is proposed to explain these data; it is shared by hu-1 with or-1, se-1, *humilis, orientalis* and *senegalensis*. The group-1 specificities which characterize the cells of hu-1 backcross hybrids are *a, b* and *c*.

The cells of se-1 backcross hybrids and *senegalensis* absorbed the agglutinins for themselves and for each other as well as for the cells of E, ch-1 and hu-1 backcross hybrids and for *guinea* and *chinensis* (table 1, columns 5 and 10). Agglutinins for se-1 backcross hybrids and *senegalensis* are absorbed by the cells of or-1 backcrosses, *orientalis* and *humilis*, hence, a fourth specificity *d* is postulated. The specificities for the cells of se-1 backcross hybrids and *senegalensis* were designated *a, b, c* and *d*.

Finally, cells from *orientalis*, some representatives of *humilis* and or-1 backcross hybrids, respectively, absorbed completely from the or-1 antiserum the agglutinins for themselves and for each other as well as for the cells of each of the other species and backcross hybrids listed in table 1. Therefore, at least five specificities must be postulated to describe the antigenic structure of the or-1 cellular antigen. The specificities for the group-1 antigens of or-1 backcross hybrids, *orientalis* and some birds of *humilis* are designated as *a, b, c, d,* and *e;* one or more of these specificities is shared by or-1 with the cells of the heterologous members of the group-1 backcross hybrids and the species given in table 1.

The method of analysis used here to ascertain the antigenic structure of the cellular antigen or-1 has also been applied to the analyses of antisera prepared against the cells of other backcross hybrids possessing the respective cellular antigens ch-1, hu-1, se-1 and E. The results of the tests with these antisera are given in tables 2, 3, 4 and 5. The antigenic specificities identified by these results are listed in the last column of each table.

If an antiserum is prepared against the cells of a species possessing a cellular antigen related to the group-1 antigens and, after absorption with the cells of *risoria* and *livia*, is used as an agglutinating reagent against the cells of the respective backcross hybrids, positive reactions will indicate the presence of agglutinins specific for group-1. Agglutination of the cells of the homologous species, or any other species, can be due to the presence of agglutinins specific for cellular antigens unrelated to group-1, therefore, the presence or absence of group-1 agglutinins in the antisera produced against the respective species was established by tests on the cells of group-1 backcross hybrids, only. It was also possible to separate the group-1 agglutinins in species antisera by absorption with the cells of the different backcross hybrids and species possessing antigens related to group-1.

The data presented in table 1 show that the specificity *a* is found on the cells of each of the species and backcross hybrids tested. In contrast, the specificity *b* is associated with each of the species and backcross hybrids, except *guinea* and backcross hybrids possessing E. The specificities *a* and *b* are considered distinct. Likewise, the specificities *c, d* and *e* exhibit dissimilar distribution patterns with respect to the various backcross hybrids and species given in table 1; each specificity is regarded as being serologically distinct from the others. As a result of the analyses described here it has

been observed that a particular specificity may belong to one of two categories. Its distribution with respect to the various species and backcross hybrids may either parallel the distribution of a previously described specificity or it may be unlike that of one previously described. Those specificities which exhibit an identical distribution are given a common designation and those which are unlike in their respective distributions have been given differentiating designations. For example, hu-1 shares specificities *a*, *b* and *c* with or-1, as previously described (table 1). If the group-1-specific antibodies in the hu-1 antiserum had the same specificities as those in the or-1 antiserum the cross-reactions, towards cells other than hu-1 and or-1, of the hu-1 antiserum absorbed with hu-1 cells (table 2) should parallel those of the or-1 antiserum absorbed with hu-1 cells (table 1). The parallelism would obtain by virtue of the antigenic specificities *a* and *b*. No evidence of an antibody for specificity *c* was demonstrable in the interaction between the hu-1 antiserum and the pertinent cells of table 2. The additional specificity *f* assigned to or-1 depended upon the reactivity of or-1 cells following absorption of the hu-1 antiserum with the cells of se-1 backcross hybrids and *senegalensis*, respectively. Additional antigenic specificities to those given in table 1 were assigned to the cells of the backcross hybrids and of the species by virtue of the same kind of comparisons of the results which are given in tables 2 to 5. Specificities which parallel each other and have been identified by means of antisera prepared against the cells of different species or backcross hybrids may be structural homologues, but this need not be the case. The different specificities which are presumed to reflect the interrelationships of the antigens of group-1, as present in the available backcross hybrids and in the parental species, are summarized in table 6.

With a single exception, the specificities identified by absorption of antisera to the different species were indistinguishable from those identified following the analysis of antisera prepared against the respective backcross hybrids. The specificity *u* (table 6) which is shared by *guinea* with backcross hybrids possessing or-1 and E, respectively, and also *humilis* and *orientalis*, was detected by means of an antibody present in an anti-*guinea* serum. (The details of the reactions will be furnished upon request.)

The absorption analysis of antisera prepared against the cells of the respective species and backcross hybrids revealed the existence of at least 21 serologically distinct components, as are listed in table 6. The 21 components

TABLE 6. *Summary of known group-1 specificities*

Cells tested	Specificities associated with the group-1 cellular antigens																			
	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u
ch-1	+*	+								+			+	+	+	+	+	+			
hu-1	+	+	+			+	+	+													
or-1	+	+	+	+	+	+							+				+				+
se-1	+	+	+	+			+			+	+	+	+	+			+	+			
E	+										+				+		+	+	+	+	+
chinensis	+	+								+			+	+	+	+	+	+			
humilis	+	+	+	+	+	+	+	+	+	+			+				+				+
orientalis	+	+	+	+	+	+	+	+		+			+				+				+
senegalensis	+	+	+	+			+			+	+	+	+	+			+	+			
guinea	+														+		+	+	+		+

* "+" refers to the presence of the particular specificity on the cells of the respective species or backcross hybrids.

may be considered a minimal number and absorption with cells from species other than those described in this paper might be expected to provide evidence for new components.

The group-1 antigen of *chinensis* and of backcross hybrids possessing ch-1 share specificities *a, b, j, m, n, o, p, q,* and *r,* and are serologically indistinguishable. The same is true of the group-1 antigens of *senegalensis* and backcross hybrids with se-1 which carry the specificities *a, b, c, d, g, j, k, l, m, n, q* and *r* and are similarly indistinguishable. In contrast, the group-1 antigens of *humilis* and the hu-1 backcross hybrids, *orientalis* and the or-1 backcross hybrids as well as *guinea* and the E backcross hybrids are dissimilar.

The hu-1 antigen of backcross hybrids possesses no components that are not also present on the cells of *humilis,* but the group-1 antigen of *humilis* has at least six specificities *d, e, j, m, q,* and *u* which are not found on the cells of hu-1 backcross hybrids. Cells from individual hu-1 backcross hybrids available during the course of these experiments were used with cells from *risoria* and *livia* to absorb an antiserum prepared against the pooled cells from a number of hu-1 backcross hybrids. It was established that the hu-1 antigen of all backcross hybrids tested exhibited no detectable serological variability and that none of the absorbed fluids contained humilis specific agglutinins. Absorption of antisera prepared against the cells of ch-1, or-1, se-1 and E backcross hybrids with the pooled cells of *risoria, livia* and *humilis* removed completely the agglutinins specific for hu-1 backcross hybrids; however, the cells of hu-1 backcross hybrids lacked the capacity to absorb the agglutinins from these antisera for cells from most, but not all, representatives of humilis available for testing. These results suggest that individual differences within *humilis* are to be expected with regard to the hu-1 character.

Since reagents specific for the cells of backcross hybrids possessing the cellular antigens ch-1, or-1, se-1 and E, respectively, contain agglutinins which differentiate the hu-1 antigen of backcross hybrids from a related cellular antigen in individuals of *humilis,* the various group-1 antisera were absorbed with cells from each of 28 representatives of *humilis.* Based on the capacity of cells from individual birds to remove antibodies for known group-1 specificities it was possible to identify at least three classes of *humilis.* It was found that the different classes of birds shared among themselves at least six specificities *a, b, c, g, h* and *i.* Classes II and III are differentiated from Class I with respect to the specificities *d, j, m* and *q.* Class III has the specificity *e* which is absent from the cells of individuals belonging to Classes I and II. The group-1 antigen of Class I individuals of *humilis* is serologically indisguishable from the group-1 antigen present on the cells of the hu-1 backcross hybrids which are currently available. The group-1 antigen of Class III birds is very closely related to the group-1 antigen of at least some individuals of *orientalis.* The absorption of an antiserum prepared against the cells of several different *humilis* with cells from each of the 28 representatves of this species resulted in the identification of Classes I and II, only. Presumably, either antibodies against the specificity *e* which differentiates Class III from Class II were not produced by the immunized animals, or the donor birds included no Class III individuals.

Some data concerning the genetic relationship of group-1 antigens belonging to Classes I and II of *humilis* are available. A family of 10 hybrid progeny was obtained from the mating: *humilis* × *risoria.* The cells of the *humilis* parent were not available for testing but cells of the offspring were tested with appropriate reagents. The tests indicated that four of the progeny belong to Class I and six to Class II. The data are consistent with the assumption that a pair of contrasting

alleles was present in the *humilis* parent of the hybrid offspring.

With respect to reagents for or-1, the cells of backcross hybrids possessing the or-1 cellular antigen and cells from *orientalis* were serologically indistinguishable (table 1). Contrasted with these findings was the observation that the cells of or-1 backcross hybrids did not absorb completely the agglutinins for the cells of three representatives of *orientalis* from reagents specific for ch-1 (table 4), hu-1 (table 2) and se-1 (table 3). The or-1 antigen of backcross hybrids has no specificities that are not also present on the cells of the *orientalis* tested, but the related antigen of the three individual *orientalis* has at least three specificities *g*, *h* and *j* which are lacking on the cells of or-1 backcross hybrids. There is no evidence for the occurrence of more than one antigenic type of or-1 on the cells of available backcross hybrids and no variants of the group-1 antigen of *orientalis* have been found within the species. However, the results cited above are readily explainable if variation in the or-1 character exists among individuals of *orientalis*.

Absorption of an anti-E reagent with cells from each of 11 representatives of the species *guinea* removed the agglutinins for the absorbing cells as well as for the cells of other *guinea*. However, each absorbed fluid contained agglutinins for cells from backcross hybrids possessing the antigen E. Further, following the absorption of an anti-se-1 reagent with the cells of *guinea*, antibodies for the absorbing cells were removed but agglutinins remained which were specific for the cells of E backcross hybrids (table 3). The observation that cells from E backcross hybrids and *guinea*, respectively, do not absorb reciprocally for each other from anti-E (table 5) and anti-se-1 (table 3) sera indicates that the E antigen of backcross hybrids possesses specificities which are absent from a related group-1 antigen which is characteristic of the 11 *guinea* whose cells were examined. The contrasting specificities are identified as *k* and *t*. Individual differences with respect to the E character presumably obtain in *guinea*, unless the above results indicate that a "hybrid substance" is associated with the E character.

Discussion and Conclusions

On the basis of genetic and serological criteria, the gene or genes which determine the cellular antigens ch-1 of *chinensis*, or-1 of *orientalis*, se-1 or *senegalensis* and probably hu-1 of *humilis* may be regarded as members of a multiple allelic series of genes in closely related species which are responsible for the determination of a serologically related group of cellular antigens (Stimpfling and Irwin, 1960). The E antigen of *guinea* has been included in this set by virtue of its serological relationship to the group-1 antigens.

The data presented here show that the cellular antigens ch-1, hu-1, or-1, se-1 and E, respectively, not only share serological properties in common but that each member of group-1 possessed at least one specificity not shared by any other members of group-1. The results have confirmed and extended earlier observations concerning the antigenic complexity of the cellular antigens ch-1 and se-1 (Irwin, 1951, 1953).

The group-1 antigens of ch-1 backcross hybrids and *chinensis*, as well as the group-1 antigens of se-1 backcross hybrids and senegalensis, are, respectively, serologically indistinguishable. However, some individuals of *senegalensis* lack the se-1 character, as was predicted because of the variation in this character noted among the species hybrids produced from matings with *risoria* (Irwin and Cole, 1945). Further, three classes of individuals with respect to antigenic specificities of hu-1 were demonstrable in *28 humilis*. Similarly, the results of these tests suggest that individual differences exist in *orientalis* with respect to the or-1 character, and in *guinea* with respect to

the E character. It seems reasonable to conclude that (a) variation is expected within a species with respect to antigenic characters which distinguish that species from one or more others and (b) that a multiple series of genes may be involved, as would be required in *humilis*.

The relationships among the causative genes in the various species have been discussed on the basis of the cellular antigens being products of two or more linked genes (Irwin, 1953). Irrespective of whether one gene, or two or more linked genes, determine the cellular antigens in each species, it is clear that the cellular antigens have multiple specificities. Comparable complexities are recognized in the cellular antigens of systems in other species, e.g., the B system of cattle (Stormont *et al.*, 1951), the H-2 system of the domestic mouse (Amos, 1959). The group-1 specificities have interlocking relationships within the species *humilis, orientalis* and *guinea*, and among the species, as well as one or more specificities which are peculiar to each species.

The possibility cannot be completely excluded that the gene or genes which determine the presence of the group-1 antigen on the cells of one parental species may as a result of interaction between allelic or non-allelic genes produce an altered product when transposed to the foreign genotype of a backcross hybrid. Whether or not this is true of the or-1 and E cellular antigens has not been established. With respect to the antisera used in these studies attempts to demonstrate the presence of a "hybrid substance" on the cells of group-1 backcross hybrids were not successful. The cells of backcross hybrids possessing E were characterized by the presence of two specificities *k* and *t* which were not present on the cells of *guinea*. It should be noted, however, that the anti-E serum described here was prepared against the cells of hybrids known to be homozygous for the allele which determines the cellular antigen E. Consequently, interaction between different alleles at the E locus does not seem possible, but non-allelic interaction cannot be excluded.

The group-1 antigens of *chinensis, humilis, orientalis* and *senegalensis* display a greater serological affinity among themselves than they do with the related group-1 antigen E of *guinea*. This would be expected on the basis of their taxonomic relationships.

The data summarized in table 6 indicate that backcross hybrids possessing hu-1 and or-1, respesctively, are differentiated by at least eight specificities but the group-1 antigens of the corresponding parental species *humilis* and *orientalis* differ by a single specificity, only. The results suggest that *humilis, orientalis* and *guinea* possess multiple alleles at the group-1 locus which determines cellular antigens exhibiting varying degrees of interrelationships within each species as well as between the five species. Presumably, the different group-1 specificities are an index of the changes in the gene or genes in each species which have taken place during the evolution from a common ancestral gene in an ancestral species.

Summary

A further analysis of the genetic and serological interrelationships of a system of red blood cell antigens of pigeons and doves is presented. The cellular antigens which have been considered include ch-1 (formerly d-1) of *Streptopelia chinensis*, hu-1 of *St. humilis*, or-1 of *St. orientalis*, se-1 (formerly s-2) of *St. senegalensis* and E of *Columba guinea*. The antigens ch-1, or-1, se-1 and probably hu-1 behave as contrasting characters in the progeny of appropriate matings (Stimpfling and Irwin, 1960) and are presumed to be determined by genes in the parental species that are allelic in evolutionary derivation. The antigen E of *guinea* has been included in the system by virtue of its serological affinities to the other members of the group.

The serological analyses show that the

respective cellular antigens not only share serological properties in common but that each of the related antigens possesses at least one specificity not shared by any of the others. The results indicate that the five cellular antigens are collectively characterized by at least 21 serologically distinct specificities. Evidence has been presented for the occurrence within the species *humilis, orientalis, senegalensis* and *guinea* of variant forms of the corresponding antigens hu-1, or-1, se-1 and E. It is presumed that the genes which determine each antigenic character form a series of multiple alleles, and that their corresponding antigens reflect the changes that have taken place in the evolution of the respective species from a common ancestor.

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DIFFERENTIAL RADIOSENSITIVITY AMONG THE PROBABLE GENOME DONORS OF BREAD WHEAT

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Radiation experiments in varieties of bread wheat (*Triticum aestivum*, 2n=42) have shown that this hexaploid plant exhibits a mass mutating effect following treatment with X-rays, neutrons and other mutagens (Gustafsson, 1947; MacKey, 1954a; Natarajan et al., 1958). The number of chromosome breaks induced in bread wheat irradiated with fast neutrons was also far in excess of the number to be expected on the basis of comparison with *T. monococcum* (2n = 14) and *T. dicoccum* (2n = 28) (Swaminathan and Natarajan, 1957). Stebbins (1957) has pointed out that the relatively higher radiosensitivity of bread wheat in comparison with the einkorn and emmer wheats may be due to the D genome of bread wheat being more radiosensitive than the other two genomes. Using chromosome breakage, DNA content of cells and viable mutation frequency as indices of radiosensitivity, Bhaskaran and Swaminathan (1960) found that while diploid and tetraploid wheats show a radiation response similar to those of diploid and autotetraploid barley, hexaploid wheat is consistently more sensitive, particularly to radiations with higher LET.

The A and D genomes of bread wheat are now known to have been derived from einkorn wheat and *Aegilops squarrosa* respectively (Kihara, 1944; McFadden and Sears, 1946). The origin of the B genome has been a subject of controversy and speculation but the recent studies of Sarkar and Stebbins (1956) and Riley et al. (1958) indicate that *Aegilops speltoides* var. *ligustica* or some related species belonging to the Sitopsis section of *Aegilops* might have been the donor of the B genome. In order to examine whether any prominent differences exist in the radiosensitivity of these probable genome ancestors of bread wheat, the present study was undertaken in *Triticum monococcum*, *Aegilops speltoides* var. *ligustica* and *Aegilops squarrosa*.

Material and Methods

The variety Japanese Early of *T. monococcum, ligustica* of *Aegilops speltoides* and a strain of *Aegilops squarrosa* obtained from the Plant Breeding Institute of Cambridge were used. The seeds were all dehusked and were equated to a uniform moisture content, prior to irradiation. X-ray treatments were carried out at 50 KV, 2mA, without filter and a target distance of 15 cms from the tube. Two dosages, 11,000 r and 16,000 r, were given and the seeds were germinated at a constant temperature. For scoring chromosome aberrations the technique of Wolff and Luippold (1956) was followed. The seeds of each X_1 plant were collected separately and used for raising the X_2 progenies.

Observations

(a) *Germination and survival:* In *T. monococcum* all seeds germinated in control and 11,000 and 16,000 r treatments. The germination was, however, affected by the X-ray treatment in both *A. speltoides* and *A. squarrosa*. At 11,000 r in *A. speltoides*, the percentage reduction in germination as compared to control amounted to 14.8. This percentage was 10.8 and 52 in *A. squarrosa* at 11,000 r and 16,000 r respectively. The germinated seedlings all survived and grew to maturity. No seed setting, however, occurred in *A. speltoides* both in the control

as well as in the irradiated plants, probably due to the unfavorable environmental conditions.

(b) *Chromosome aberrations:* The types and frequencies of chromosome aberrations induced by the different treatments were scored in metaphase plates in root tips fixed immediately after the germination of seeds. Mitosis was regular in the root tips fixed from control seeds. In the irradiated seeds, however, various abnormalities such as deletions, interchanges, dicentrics, rings and minutes were observed. Single- and two-hit aberrations were scored independently but were pooled (taking each two-hit aberration as two single-hit ones) for the purpose of calculating the total number of breaks observed in a cell. All cells which could be clearly analyzed were scored. The data are given in table 1. It will be observed from the data that *A. squarrosa* had the highest frequency of chromosome aberrations per cell.

The total lengths of the somatic chromosome complements of the varieties of *T. monococcum, A. speltoides* and *A. squarrosa* used in this study, as determined in strictly comparable preparations, were 127.13, 157.50 and 90.25 microns respectively. The number of chromosome aberrations per 100 microns of chromosome length was determined in all the three species (fig. 1) and the data were subjected to an analysis of variance test. The test showed that the differences in the frequency of aberrations observed between different species is significant. The results therefore suggest that radiosensitivity as measured by the frequency of aberrations per unit length of chromosome increases in the order *A. speltoides, T. monococcum* and *A. squarrosa.*

TABLE 1. *Frequency of chromosome aberration*

Species	X-ray dose in r units	No. of cells analyzed	Mean no. of breaks per cell ±S.E.
T. monococcum	11,000	428	0.775 ± 0.07
	16,000	440	0.905 ± 0.073
A. speltoides	11,000	39	0.69 ± 0.19
	16,000	347	0.735 ± 0.072
A. squarrosa	11,000	338	0.81 ± 0.12
	16,000	367	1.05 ± 0.075

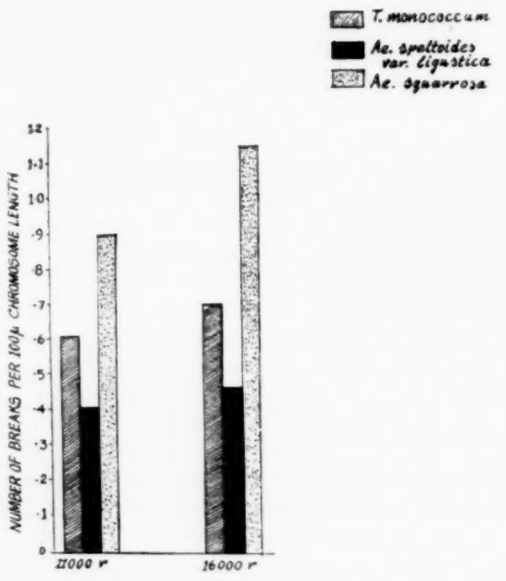

FIG. 1. Number of aberrations observed per 100 μ of chromosome length.

(c) *Chlorophyll mutations in the X_2 generation:* The seeds from individual control and X_1 plants of *T. monococcum,* and *A. squarrosa* were sown in separate pans for studying the frequency and types of chlorophyll mutations occurring in the two treatments. While no chlorophyll mutations occurred in the control population, several such mutants were found in the progenies of material treated with 11,000 and 16,000 r of X-rays. The data are given in table 2. The 11 mutants observed in *T. monococcum* treated with 11,000 r comprised 9 *albinas* and 2 *tigrinas.* In the *tigrina* type, there were transverse bands at uniform intervals with pink color alternating with green. In the 16,000 r treatment, 50 chlorophyll mutants were recorded out of which 47 were *albinas,* 1 *striata* and 2 *tigrinas.* The *tigrina* and *striata* types were viable, while the *albina* mutation was lethal in all cases. Only *albina* mutants occurred in *A. squarrosa,* the frequency of their occurrence being much higher than that observed in *T. monococcum.* In both *T. monococcum*

TABLE 2. *Frequency of chlorophyll mutations observed in the X_2 generation*

Species	Treatment	No. of X_2 plants scored	No. of chlorophyll mutants observed	Percentage of X_2 families segregating for chlorophyll mutation
T. monococcum	Control	2200	0	0
	11,000 r	316	11	22.2
	16,000 r	263	50	16.6
A. squarrosa	Control	10	0	0
	11,000 r	211	28	51.8
	16,000 r	130	14	36.4

and *A. squarrosa*, the chlorophyll mutation rate was higher at 11,000 r in comparison with 16,000 r, thereby indicating a greater degree of inviability of aberrant gametes at the higher dosage.

DISCUSSION

The results of the present study indicate that *A. squarrosa* is relatively more radiosensitive than *T. monococcum*, when either the frequency of chromosome aberration or chlorophyll mutation is used as an index of sensitivity. *A. squarrosa* is also more sensitive in comparison with *A. speltoides* var. *ligustica* with regard to the frequency of chromosome aberrations. Further studies are in progress to find out whether the higher frequency of chromosomal aberrations found in *A. squarrosa* is due to a greater initial damage or due to other factors such as a less rigid diplontic selection of cells and a slower rate of reunion due possibly to a reduced rate of protein synthesis in the germinating seeds. The chlorophyll mutation rate could not be calculated in *A. speltoides* due to the complete failure of seed setting in this species in New Delhi. Factors such as moisture content of seeds, temperature at the time of irradiation and germination, dose rate, stage and time of fixation of root tips, etc., which can introduce differences in the observed radiation effects, were kept as uniform as possible. The indications obtained could therefore be considered to be of at least some validity in the varieties studied.

Chromosome length has been considered to be an important factor with regard to radiation response, the longer chromosome species being usually more sensitive to radiation than the smaller ones (see Östergren et al., 1958, for a recent discussion of this subject). Though all the three species used in the present study have $2n = 14$, the total chromosome length is much less in *A. squarrosa* in comparison with the other two species. The higher radiosensitivity of this species is hence somewhat surprising.

It is now known that the ratio of total chromosome length in diploid, tetraploid and hexaploid wheats is not of the order 1:2:3 but is about 1:1.6:2.0 (Marshak and Bradley, 1944; Swaminathan and Natarajan, 1957; Bhaskaran and Swaminathan, 1960). Inferences drawn from the length of metaphase chromosomes are, however, subject to the limitation that the observed differences in length may be due to differential condensation. The data of Bhaskaran and Swaminathan (1960) concerning the DNA content of cells of some varieties of *T. monococcum*, *T. dicoccum* and *T. aestivum* are hence of interest. The DNA content in these three species varied in the ratio 1:1.45:1.92. There is thus a close similarity between the values obtained by measuring chromosome length and DNA content and it seems justifiable to use metaphase chromosome length to estimate the total length of the chromosome complement in this material. The total length of the chromosome complements of *T. monococcum*, *Aegilops speltoides* var. *ligustica* and

Aegilops squarrosa varied in the ratio 1:1.239:0.710. Sears (1959) has assigned each chromosome of the bread wheat variety Chinese Spring to its respective genome and he (Sears, 1954) has also calculated the length of each of the 21 chromosomes. The proportion of the total lengths of the chromosomes belonging to the A, B and D genomes, as calculated from his data, falls into the ratio 1:1.217:0.838. Thus, the proportions of the lengths of the total chromosome complements of *T. monococcum, A. speltoides* var. *ligustica* and *A. squarrosa* and that of the A, B and D genome chromosomes of Chinese Spring show a very close similarity, lending further support to the view that these three species are the donors of the A, B and D genomes respectively. However, the total chromosome length and DNA content of varieties of bread wheat are only twice as great as that of *T. monococcum,* and not three times as would be expected from considerations of chromosome number and the additive value of the total chromosome length of the three genome donors. It hence appears that chromosome diminution has occurred in the tetraploid and hexaploid wheats during their evolution. Our data suggest that the diminution has not been restricted to the chromosomes of any particular genome but has taken place uniformly in the chromosomes of all the three genomes.

In mutation studies in bread wheat, it has been found that chlorophyll-deficient types are extremely rare (Stadler, 1930; MacKey, 1954b; Natarajan et al., 1958). None of the 21 nullisomics in bread wheat show chlorophyll deficiency and obviously the buffering effects of duplicate loci minimize the chances for the expression of mutations. On the other hand, the frequency of viable, morphological mutations has been found to be very high by several workers as can be seen from the data in table 3.

On basis of such data MacKey (1954a) has remarked that the skepticism of Stadler (1930) with regard to the suitability of polyploid plants in mutation research

TABLE 3. *Frequency of viable mutations observed in varieties of bread wheat*

Variety	Mutagen	Mutation rate (% of mutants per X_1 plant progeny)	Reference
Winter wheat (Scandia III)	X-rays	76.8	MacKey, 1954b
Spring wheat (Rival)	X-rays (different dosages)	21.5–53.0	MacKey, 1954b
Spring wheat (Rival)	Fast neutrons (different dosages)	34.0–167.0	MacKey, 1954b
C-591	32_p	42.4	Pal et al., 1958
	35_s	94.0	Pal et al., 1958
	X-rays (different dosages)	22.2–53.5	Pal et al., 1958
	Fast neutrons	21.5	Pal et al., 1958
	Groundnut oil	155.5	Pal et al., 1958
	Castor oil	61.0	Pal et al., 1958
	X-rays	86.4	Bhaskaran and Swaminathan, 1960
	Thermal neutrons	129.1	Bhaskaran and Swaminathan, 1960
Brescia	Thermal neutrons	9.70–33.15	D'Amato & Moschini, 1959
	X-rays	7–26.53	D'Amato & Moschini, 1959

is no longer tenable. In contrast to bread wheat, viable mutation frequency in the tetraploid emmer wheats is very low. Tetraploid wheat has been found to resemble autotetraploid barley in this respect (Bhaskaran and Swaminathan, 1960). A combination of the effects of duplicate factors at many loci and of functional diploidy in others seems to give rise to a high viable mutation rate in hexaploid wheat. The restricted spectrum of the induced morphological mutations coupled with a high frequency of occurrence of each category of mutant would support this view. Besides giving rise to a higher frequency of viable mutations, bread wheat also shows a relatively higher chromosomal radiosensitivity in comparison with tetraploid wheat. Though this may in fact be due to differences in the viability of affected cells, the results of the present study would suggest that other factors such as genomic differences in radiosensitivity may also have a role.

Differences in radiosensitivity among different plant species and even among varieties of the same species have been demonstrated in several cases (Smith, 1942; Lamprecht, 1956; Blixt, et al., 1958) and it may hence be misleading to extrapolate the data obtained by us from the probable ancestors of bread wheat to explain a situation existing in the present-day varieties of this important cereal which has been the object of a rigorous selection pressure for a very long time. It is, however, tempting to suggest from our results that the *squarrosa* genome may confer upon the hexaploid wheat a physiological state that leads to more breakage of all the chromosomes and thereby to a higher degree of chromosomal radiosensitivity and mutability.

Summary

A study of comparative radiosensitivity was undertaken in *T. monococcum*, *Aegilops speltoides* var. *ligustica* and *Aegilops squarrosa* which are now known to be the probable donors of the A, B and D genomes respectively of *T. aestivum*. Two doses of X-rays, 11,000 and 16,000 r, were given and survival, frequency of aberrations per unit length of chromosome and X_2 chlorophyll mutation rate were used as indices of radiation sensitivity. By all these three criteria, *A. squarrosa* was found to be much more radiosensitive than the other two species. Since bread wheat has been found by several authors to be more radiosensitive in comparison with einkorn and emmer wheats, it seems likely that the *squarrosa* genome might have introduced into bread wheat a physiological state which promotes the incidence of a higher frequency of chromosomal aberrations and viable mutations following treatment with mutagens. The results support the view of Stebbins (1957) that in an allopolyploid plant, differences in genome sensitivity may complicate the analysis of the relationship between polyploidy and radiosensitivity.

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MEIOTIC DRIVE IN NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER*. III. POPULATIONAL IMPLICATIONS OF THE SEGREGATION-DISTORTER LOCUS[1,2]

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If, among the successful gametes from heterozygotes, one allele is regularly included in more than half, it may increase in frequency even if it has a harmful effect. Unequal gamete production, when attributable to the mechanics of meiosis, has been called meiotic drive (Sandler and Novitski, 1957). An example is segregation-distortion in *Drosophila melanogaster*, the cytogenetic behavior of which has been reported by Sandler, Hiraizumi and Sandler (1959) and Sandler and Hiraizumi (1959).

The observations that are relevant to this discussion are: 1. Segregation-distortion depends on a locus named *Segregation-distorter* (*SD*), which is located in the centromeric heterochromatin of chromosome II. 2. Males heterozygous for *SD* and a normal chromosome transmit the *SD*-carrying chromosome in great excess; this excess is not due to post-zygotic mortality, nor is there any reduction in the fertility of *SD*/+ males. 3. Segregation in *SD*/+ females is normal. 4. It appears necessary, in order for segregation-distortion to occur, for the *SD* locus to synapse with the SD^+ allele on the homologous chromosome; for, if the SD^+-bearing chromosome is structurally abnormal in the *SD* region, the distortion is reduced and usually eliminated altogether. 5. Males homozygous for *SD* have normal segregation ratios.

The purpose of this paper is (1) to show that segregation-distortion does in fact occur in nature and that the population consequences are qualitatively in agreement with expectations from the theory of meiotic drive; (2) to show that the results from experimental populations bear out the quantitative predictions insofar as these have been tested; (3) to present a mathematical treatment of the population consequences of some special cases of meiotic drive with special emphasis on those phenomena which bear on the behavior of *SD*; and (4) to consider the particular way in which one population of Drosophila has minimized the detrimental effects of the spread of *SD*.

SD IN NATURAL POPULATIONS

In a collection of Drosophila, made in 1956 from a natural population in Madison, Wisconsin, six chromosomes bearing the *SD* locus were recovered in a total of 183 second chromosomes tested. Among these six *SD*-bearing chromosomes, five also carried a recessive lethal. Allelism tests revealed that the lethal carried by these five chromosomes was the same. Moreover, salivary gland examination showed that the five lethal-bearing chromosomes carried two inversions in the right arm of chromosome II, a small proximal and a large distal inversion, which together cause almost complete linkage of the *SD* gene, the lethal, and the two inversions. It has so far been impossible to separate the proximal inversion from the lethal. The remaining lethal-free *SD* line contains only the more distal of the two inversions and also exhibits a reduced frequency of crossing over (although higher than in the other lines). We designate the doubly inverted lines as *SD-5* type, the

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lethal-free singly inverted line as *SD-72* type.

The high frequency of *SD* in this population, since it is in most instances inseparable from a lethal, is a direct demonstration that segregation-distortion is effective in maintaining gene frequencies against opposing selection. Indeed, the homozygous *SD* locus itself causes a reduced viability as is shown by the fact that the lethal-free *SD-72* line has a larval viability of only 68 per cent that of Canton-S, a standard laboratory inbred stock.

In 1958, another wild collection was made in Madison approximately three miles from the site of the original collection. Again, *SD*-bearing chromosomes were recovered. Unfortunately, collections could not be made at the original site because building construction had spoiled the location for Drosophila collecting. In the 1958 collection, *SD*-bearing chromosomes of at least two types were recovered. One of these appears to be indistinguishable from the *SD-5* type. Among the others several have given a crossover pattern different from either the *SD-5* or *SD-72* types. The distal inversion of *SD-72* permits crossing over only proximal to it, while the two inversions of *SD-5* permit crossovers only distal to both. The new type has both proximal and distal crossovers. The reason for supposing that this is not the same as the proximal inversion of *SD-5* is that it contains no lethal.

Since the new collection was made near the home of one of us (JFC), it is possible that the *SD-5* type is descended from laboratory escapees. The fact that a new inversion type appears to be present makes it very unlikely that all the *SD*'s are from this source. It is, however, completely clear that the *SD* locus on these new chromosomes is dentical with the *SD* loci previously recovered by all criteria applied. These are: 1. The locus is able to exhibit the phenomenon of segregation-distortion. 2. *SD* from the original collection does not lead to distorted ratios when heterozygous with the newly recovered *SD*-bearing chromosomes. 3. The new *SD* alleles are also located near the centromere. We designate these new *SD*-bearing chromosomes as *SD-100* type, although several types may actually be included.

From the foregoing we see that all of the *SD* loci recovered in nature have been associated with inversions which are extremely efficient crossover suppressors for the right arm of chromosome II. Crossover data from all of the tested *SD* lines are given in table 1.

The invariable association of *SD* with inversions is a surprising observation, since it is well known that inversions are relatively rare in wild populations of *D. melanogaster* (Dubinin, *et al.*, 1937; Ives, 1947). It thus appears most probable that the inversions are somehow maintained because of the presence of *SD*. It is, in fact, clear from previously reported experimental results (Sandler and Hiraizumi, 1959) that this is true. For the rare recombinant chromosomes containing *SD* all give less distorted ratios than the original chromosomes from which they were derived.

As stated earlier, the relative viability of the lethal-free homozygous *SD-72* line is about two-thirds of normal. This, of course, is the viability under nearly ideal laboratory conditions, and it seems

TABLE 1. *Frequencies of various recombinant types from the cross* SD(*In*)/cn bw ♀ ♀ ×cn bw ♂ ♂

SD type	Non-crossover	Constitution of crossovers				Total
		SD bw	*cn* In	*SD* In *bw*	*cn*	
SD-72	7,535	9	5	0	0	7,549
SD-5	9,305	0	0	1	0	9,306
*SD-5**	1,246	0	0	4	5**	1,255

* This high rate of crossing over was observed in this experiment only and not in tests of the inversion-bearing recombinants from this line. This result is not understood at present.

** One of these *cn* recombinants was lost before tests could be made. It is included in this class because of the identity of the other four *cn* recombinants.

reasonable that in nature *SD-72* might be effectively lethal or semi-lethal. On the other hand, it appears that the *SD-5* type lines, although completely lethal when homozygous, have, on the average, a higher and more stable distribution of k values (k = proportion of functional *SD* gametes from a heterozygote). Therefore, it might be that the *SD-5* type lines accumulate more rapidly than *SD-72* in a population. It could be, alternatively, that *SD-72* is an *SD-5* type that had recently lost the lethal and the associated proximal inversion and was, at the time of the collection, in the process of replacing the *SD-5* chromosomes. This matter will be considered again in succeeding sections.

One of the most interesting and important observations from the original 1956 collection was the following. Among the normal, SD^+, chromosomes in the population, most of those tested have been partially "insensitive" to the distorting action of *SD*. When heterozygous *SD-5* males are tested with the "standard" *cn bw* tester stock, the average k value is almost invariably higher than 0.95. When, however, a sample of three wild chromosomes collected, in 1956, from the Madison population from which *SD* was recovered were tested, the average k values were 0.87, 0.85, and 0.78 (Sandler, Hiraizumi and Sandler, 1959). Although the number of chromosomes so examined is small, it is still clear that a high proportion of second chromosomes in this population are relatively insensitive to the distorting action of *SD*. It is, to be sure, true that most laboratory stocks carry second chromosomes which are also relatively insensitive to *SD*. But on the other hand, five populations collected in nature from various parts of Japan were tested for the presence of *SD* loci and also for their sensitivity to *SD-5*. Here it was found that none of these five populations contained *SD* nor did any contain insensitive alleles. In fact, the sensitivity of the Japanese wild type strains was at least that of the standard tester stock. The results of such tests and the k values for these Japanese wild type lines are given in table 2.

TABLE 2. *Tests of the sensitivity of second chromosomes in five Japanese populations and the Madison population. The cross is*

SD-5/Cy ♀ ♀ × SD-5/+ ♂ ♂

Source of + chromosome	Phenotype of progeny		
	Cy	+	k*
Hayahoshi	453	4	0.99
Ishima	589	10	0.98
Otaru	685	3	0.99
Tokyo	461	0	1.00
Uwajima	683	4	0.99
Madison	1,002	166	0.83

* $k = 1 - 2r/1 - r$ where r is the ratio of wild progeny to total progeny.

Although it is clear that the need for a further study of the geographical distribution of *SD* loci and insensitive SD^+ alleles remains, there is, from these data, at least the indication that there may be an association between the presence of the *SD* alleles and insensitive SD^+ alleles. This association could come about, perhaps, because insensitive SD^+ alleles arise spontaneously and accumulate in populations because of the presence of *SD*, or it might be that insensitive alleles arise only by some process that depends on the presence of the *SD* allele (Sandler and Hiraizumi, 1959). This point will be developed below.

MATHEMATICAL RESULTS

SD can exist in a variety of states, each of which is recognizable by a characteristic distribution of k values. Furthermore, these states can be induced by appropriate mating schemes. For example, there are *SD* alleles which produce, from individual test males, a distribution of k values which is essentially flat and ranges from no distortion to k values approaching unity.

Even alleles which are stable produce k values differing from those observed when heterozygous with the "standard

tester" chromosome depending on the sex of the parent contributing the *SD* allele to the tested male ("conditional distortion") and depending on the SD^+ allele in the *parent* of the tested male.

Additional complications arise because it is found that SD^+ alleles exist in a variety of states each of which has a characteristic k distribution when tested with some standard tester *SD* chromosome. These states of SD^+, furthermore, are also modifiable by genetic manipulation.

For these reasons, a completely general theory of meiotic drive would appear to be difficult in the extreme, if not impossible. Consequently, we content ourselves here with mathematical treatments of special and necessarily simplified cases.

An important characteristic of meiotic drive in cases so far reported is that its effect is restricted to one sex. This causes complications in the mathematical treatment. Therefore we shall start with a simple model in which meiotic drive operates in both sexes in the same way, and then consider the case where the effect is different in the two sexes.

Meiotic Drive in Both Sexes

Sandler and Novitski (1957) showed that a driven element, which arises in a population, will increase in frequency if $2W_{01}k > 1$, where W_{01} is the fitness of the heterozygote and k is the segregation ratio as before. This can be shown as follows:

Let A_0 and A be a driven element and its normal alternative respectively, and W_{00}, W_{01} and 1 be the fitness of A_0A_0, A_0A and AA. Then

$$\Delta q = \frac{q(1-q)[q(W_{00} - 2W_{01} + 1) + (2W_{01}k - 1)]}{\bar{W}}$$

or, equivalently,

$$\Delta q = \frac{-(2W_{01} - W_{00} - 1)q(1-q)(q - \hat{q}),}{\bar{W}}$$

where

$$\hat{q} = \frac{2W_{01}k - 1}{2W_{01} - W_{00} - 1}, \qquad \bar{W} = W_{00}q^2 + 2q(1-q)W_{01} + (1-q)^2$$

and q is the frequency of A_0. At the time A_0 appears in the population we may take $q \to 0$ and for $\Delta q > 0$ we must have $2W_{01}k - 1 > 0$. At equilibrium, $\Delta q = 0$ which leads to $\hat{q} = \frac{2W_{01}k - 1}{2W_{01} - W_{00} - 1}$, where $\hat{q}$, the equilibrium frequency, is necessarily between 0 and 1.

(1) If $(2W_{01} - W_{00} - 1) > 0$ the equilibrium is stable because Δq is positive when $q < \hat{q}$ and negative when $q > \hat{q}$. Therefore the condition for a stable equilibrium is, $2W_{01} - W_{00} - 1 > 2kW_{01} - 1 > 0$ or $1 - \frac{W_{00}}{2W_{01}} > k > \frac{1}{2W_{01}}$. When $k \geqslant 1 - \frac{W_{00}}{2W_{01}} > \frac{1}{2W_{01}}$ (the right hand side of the inequality comes from $2W_{01} - W_{00} - 1 > 0$), Δq is positive for all values of $q < 1$ and this is the condition for fixation of the driven element.

(2) If $(2W_{01} - W_{00} - 1) < 0$, the equilibrium is unstable and the condition for this is, $2W_{01} - W_{00} - 1 < 2kW_{01} - 1 < 0$ or $1 - \frac{W_{00}}{2W_{01}} < k < \frac{1}{2W_{01}}$. A_0 is fixed

if $q_0 > \hat{q}$ and is lost if $q_0 < \hat{q}$ where q_0 is the initial frequency of A_0. When $\hat{q} \leqslant 0$, the driven element will be fixed regardless of its initial frequency (supposing $q_0 \neq 0$), and the condition for this is $k \geqslant \frac{1}{2W_{01}} > 1 - \frac{W_{00}}{2W_{01}}$. This is another condition for fixation of A_0.

(3) If $(2W_{01} - W_{00} - 1) = 0$, A_0 is fixed when $k > \frac{1}{2W_{01}} \left(= 1 - \frac{W_{00}}{2W_{01}} \right)$ and is lost when $k < \frac{1}{2W_{01}} \left(= 1 - \frac{W_{00}}{2W_{01}} \right)$. There is no frequency change when $k = \frac{1}{2W_{01}} = 1 - \frac{W_{00}}{2W_{01}}$.

Under random mating the effect of a driven element is always to reduce the average population fitness, unless, of course, the element itself has some other effect that is beneficial. This can be shown as follows:

The average fitness of the population is

$$\bar{W} = q^2 W_{00} + 2q(1 - q) W_{01} + (1 - q)^2.$$

When $\frac{d\bar{W}}{dq} = 0$, $q_{\max} = \frac{W_{01} - 1}{2W_{01} - W_{00} - 1}$, which is equal to $\hat{q}$ only when $k = \frac{1}{2}$.

With inbreeding the situation may be different. For example, if the *SD* locus is heterotic, there are some values of $k > \frac{1}{2}$ that give a fitness greater than for $k = \frac{1}{2}$.

Another interesting question arises if a new normal element appears in the population which is partially or completely insensitive to the driven effect. If the new element is otherwise neutral, it will have an advantage relative to the remaining normal elements and may replace all of them.

To consider this, let A_0 and A_i $(i \neq 0)$ be a driven element and its alternatives having gametic frequencies q_0 and q_i. Let the fitness of A_0A_i and A_iA_j $(j, i \neq 0)$ be W_{0i} and 1 and the segregation ratio of A_0A_i be k_i respectively. Then

$$\Delta q_i = \frac{q_i}{\bar{W}} [2q_0 W_{0i}(1 - k_i) + (1 - q_0)] - q_i.$$

Let

$$q_i = \phi_{ij} q_j (i, j \neq 0)$$

then

$$\frac{dq_i}{dt} = \phi_{ij} \frac{dq_j}{dt} + q_j \frac{d\phi_{ij}}{dt}$$

and we get

$$\frac{d\phi_{ij}}{dt} = \frac{2q_i q_0}{q_j \bar{W}} [W_{0i}(1 - k_i) - W_{0j}(1 - k_j)].$$

Here the sign of $\frac{d\phi}{dt}$ is determined by the sign of $[W_{0i}(1 - k_i) - W_{0j}(1 - k_j)]$. Therefore the A_i $(i \neq 0)$ which has the largest value of $W_{0i}(1 - k_i)$ will replace the remaining A_j's $(j \neq 0, j \neq i)$. Consider the A_i which has the maximum value of $W_{0i}(1 - k_i)$. As t goes to infinity, its frequency approaches $1 - q_0$ and it is seen that $\frac{dq_i}{dt} \to \frac{q_0(1 - q_0)}{\bar{W}} [q_0(2W_{0i} - 1 - W_{00}) - (2W_{0i}k_i - 1)]$, where W_{00} is the fitness of A_0A_0, as in the two allele case discussed earlier.

It would appear at first sight as if the introduction and accumulation of an insensitive element would increase the population fitness. Although this would usually be the case, it is not invariably so. For example, if the insensitive element results in an increase in fitness when heterozygous with the driven element, it may replace the existing sensitive element and yet cause a reduction in population fitness at the new equilibrium. The following is a numerical example.

(1)	A_0 and A_1	$W_{00} = 0$	$k_1 = 0.9$	$W_{01} = 0.6$
(2)	A_0 and A_2	$W_{00} = 0$	$k_2 = 0.8$	$W_{02} = 1.0$

The value of $W_{01}(1 - k_1)$ is 0.06; $W_{02}(1 - k_2)$ is 0.20. Therefore A_2 replaces A_1. In case (1) $\hat{q}_0 = 0.4$ while in case (2) it is 0.6. The equilibrium average fitnesses, $\bar{W}$, are 0.648 and 0.640, respectively.

Meiotic Drive Different in the Two Sexes

We now consider the case in which the k value is different in the two sexes. A special case has already been worked out by Bruck (1957), but here we are more general.

Let D and d be a driven element and its normal alternative having a segregation ratio k_1 in males and k_2 in females. Let p and P be the gametic frequency of D in males and females respectively. Let W_{00}, W_{01} and 1 be the fitness of three genotypes DD, Dd and dd in either sex, and let

$$p = \phi P$$

In the next generation,

$$\phi' = \frac{W_{00}pP + k_1W_{01}[p(1 - P) + P(1 - p)]}{W_{00}pP + k_2W_{01}[p(1 - P) + P(1 - p)]},$$

where ϕ' is the ϕ in the next generation.

We shall consider here the important special case in which $W_{00} = 0$ (i.e., homozygous D is lethal). Substituting in the above equation,

$$\phi = \frac{p}{P} = \frac{k_1}{k_2}.$$

This relation is true not only at equilibrium, but also in any generation except, of course, the first. Therefore, we take

$$\phi_{(t)} = \frac{k_1}{k_2}.$$

Let

$$\psi = \frac{1 - p}{p} \quad \text{and} \quad \eta = \frac{1 - P}{p} \quad (\psi \text{ and } \eta \text{ are dependent}).$$

Then we have

$$\psi' = \frac{\psi\eta + W_{01}(1 - k_1)\left(\eta + \frac{k_2}{k_1}\psi\right)}{W_{01}k_1\left(\eta + \frac{k_2}{k_1}\psi\right)},$$

where ψ' is the ψ in the next generation.

Putting $\eta = \psi + \left(1 - \frac{k_2}{k_1}\right)$, we have

$$\Delta\psi = \frac{\psi^2[1 - W_{01}(k_1 + k_2)] + \psi\left[W_{01}\left\{(1 - k_1)\left(1 + \frac{k_2}{k_1}\right) - (k_1 - k_2)\right\} + \left(1 - \frac{k_2}{k_1}\right)\right] + W_{01}(1 - k_1)\left(1 - \frac{k^2}{k_1}\right)}{W_{01}k_1\left(\eta + \frac{k_2}{k_1}\psi\right)}.$$

Without losing generality, we may assume $k_1 > k_2$. It can then be shown that:

(1) If $1 - W_{01}(k_1 + k_2) > 0$ or $W_{01} < \frac{1}{k_1 + k_2}$, $\Delta\psi$ is always larger than zero for any values of p and P. This implies $\psi_{(t)}$ goes to infinity as t goes to infinity, or since $\psi = \frac{1 - p}{p}$, p must go to zero and D is eliminated.

(2) If $W_{01} > \frac{1}{k_1 + k_2}$, $\Delta\psi$ is positive if

$$\psi^2[W_{01}(k_1 + k_2) - 1] - \psi\left[W_{01}\left\{(1 - k_1)\left(1 + \frac{k_2}{k_1}\right) - (k_1 - k_2)\right\} + \left(1 - \frac{k_2}{k_1}\right)\right] - W_{01}(1 - k_1)\left(1 - \frac{k_2}{k_1}\right) < 0.$$

Hence ψ increases when

$$\frac{b - \sqrt{b^2 + 4ac}}{2a} < \psi < \frac{b + \sqrt{b^2 + 4ac}}{2a},$$

where

$$a = W_{01}(k_1 + k_2) - 1 > 0;$$

$$b = W_{01}\left\{(1 - k_1)\left(1 + \frac{k_2}{k_1}\right) - (k_1 - k_2)\right\} + \left(1 - \frac{k_2}{k_1}\right);$$

$$c = W_{01}(1 - k_1)\left(1 - \frac{k_2}{k_1}\right) > 0.$$

Since $\frac{b - \sqrt{b^2 + 4ac}}{2a} < 0$ and $\frac{b + \sqrt{b^2 + 4ac}}{2a} > 0$ for any value of b, this must imply that ψ increases when ψ is smaller than a certain positive constant, $\bar{\psi}$, determined by W_{01}, k_1 and k_2, and decreases when ψ is larger than this constant. Therefore there is a stable equilibrium with the frequency $\bar{\psi} = \frac{b + \sqrt{b^2 + 4ac}}{2a}$.

Since $\bar{\psi} = \frac{1 - \hat{p}}{\hat{p}}$, we get the equilibrium frequencies of p and P, i.e.,

$$\hat{p} = \frac{1}{1 + \bar{\psi}}, \qquad \hat{P} = \frac{k_2}{k_1(1 + \bar{\psi})}.$$

SD IN EXPERIMENTAL POPULATIONS

It has now been shown that meiotic drive, considering the special case of segregation-distortion, can operate in nature, and some of the theoretical consequences in populations have been developed. We now consider the behavior of *SD* in experimental populations.

Five large population cages containing *SD* were started with the following initial constitutions: (A) 100% *SD-5*/Canton-S heterozygotes; (B) 6% *SD-5*/Canton-S heterozygotes and 94% Canton-S homozygotes; (C) 100% *SD-72*/Canton-S heterozygotes and (D) 28% *SD-72*/Canton-S heterozygotes and 72% Canton-S homozygotes; and, finally (E) 4% *SD-72*/*cn bw* heterozygotes and 96% *cn bw* homozygotes.

The wild-type chromosome in these population cages was Canton-S for the reason that this is a well-known, highly inbred, laboratory strain, and also because it shows a degree of sensitivity similar to those chromosomes collected from the same Madison population as were the *SD* alleles themselves. Samples were taken from these cages at intervals over the test period and the frequency of *SD* and the average *k* value were determined at each sampling. These results are given in table 3.

The method of sampling was as follows. Dishes containing medium were placed in the cages for two days and the adults which developed from the deposited eggs were collected. For cages A and B, males so collected were individually crossed to *SD-5*/*Cy* females and, from the ratio of *Cy* to non-*Cy* F_1, they could be classified as being *SD*/+ or +/+. The *k* values for each *SD*/+ males was then computed (see footnote to table 2). For cages C and D, collected females were individually crossed to males of the standard tester *cn bw* stock, and a single F_1 male from each such cross was tested for distortion. This gives an estimate of the frequency of *SD* only; the *k* values are of less interest since they reflect the sensitivity of the *cn bw*, not the Canton-S, SD^+-bearing chromosomes. For cage E, the *SD* locus is marked by cn^+ (see Sandler

TABLE 3. *The results from population cages containing* SD-*bearing chromosomes*

Initial constitution (the + chromosomes are Canton-S)							
Cage A:	*SD*-5/+ 100 pairs;			+/+	0 pairs;		
Cage B:	*SD*-5/+ 48 pairs;			+/+	752 pairs;		
Cage C:	*SD*-72/+ 100 pairs;			+/+	0 pairs;		
Cage D:	*SD*-72/+ 64 pairs;			+/+	164 pairs;		
Cage E:	*SD*-72/*cn bw* 32 pairs;			*cn bw*/*cn bw*	772 pairs;		

Days after construction	Gene frequency of *SD* in zygotes in cage					Average *k* value in cage	
	A	B	C	D	E	A	B
0	0.50	0.03	0.50	0.14	0.02	(0.80)	
22	0.22	0.07	—	—	—	0.76	0.78
56	0.18	0.06	—	—	—	0.71	0.80
97	0.12	—	—	—	—	0.77	—
175	—	—	0.36	—	—	—	—
196	—	—	—	0.33	—	—	—
212	0.11	0.10	—	—	—	0.74	0.68
303	—	—	—	—	0.76	—	—

	Cages A and B	Cages C and D	Overall average
Average for last sample	0.11	0.35	$k = 0.75$

and Hiraizumi, 1959) so that estimates of the gene frequencies are direct. However, the reduction in viability of homozygous *cn bw* individuals, owing to homozygosity for these mutant alleles, makes this frequency less meaningful.

We may note that in the last samples, the final frequency of *SD* is nearly the same, whether the initial frequency was high or low suggesting that the cages had been kept long enough to have attained approximate equilibrium. This observed equilibrium is 0.10 and 0.11 in the two *SD-5* cages. A hypothetical population with $k = 0.75$ for males and 0.50 for females, and a 12 percent reduction in fitness of the *SD-5* heterozygote would reach an equilibrium frequency of 0.12. (See preceding section for methods.) These are, in fact, the measured k values, and the assumption of a six percent reduction in viability due each to the *SD-5* allele and to the associated lethal is reasonable in view of the results of Hiraizumi and Crow (1960). Thus the behavior of experimental populations is, roughly at least, predictable from the theory.

For those cages containing *SD-72* as a source of *SD*, we have considerably less independent information. We do not know the average k value of *SD-72* against Canton-S, nor the viability of homozygotes and heterozygotes under cage conditions. We see, however, that *SD-72* does maintain a high equilibrium frequency of *SD*; higher, indeed, than the *SD-5* type lines. This makes it rather surprising that, in nature, *SD-72* had a much lower frequency than *SD-5* (if the small 1956 Madison population is representative). This suggests that, among the various explanations of the relative frequencies of *SD-5* and *SD-72*, the most likely is that the *SD-72* type had just arisen, as a derivative of the *SD-5* type, and had not, at the time of collection, yet replaced *SD-5*.

It was mentioned previously that an important observation in the natural populations was that a high proportion of the non-*SD*-bearing, structurally normal, second chromosomes were relatively insensitive to the distorting action of *SD*. There are two possibilities to explain this: (1) insensitive alleles arise spontaneously and, in populations containing *SD*, have a positive selective value (according to the theory presented in the previous section), or (2) the *SD* locus itself (or an insensitive SD^+ allele) produces insensitivity in normal alleles. Although it certainly is true that induction of one allele by another is an unlikely proposition, for the specific case of *SD* this may not be a real objection because such inductions are common in the *SD* system and have been found in a wide variety of genetic circumstances. Such changes are called "translocal modifications" (Sandler and Hiraizumi, 1959). The cage of interest in this connection is that containing *SD-72* and the standard tester *cn bw* chromosome. The reason that this is of particular interest is that the *cn bw* chromosome introduced there has been subjected to tests for sensitivity many times in the course of experiments on *SD*, and has been found to be completely sensitive. For this reason, a sample of *cn bw* chromosomes was collected from the *SD-72/cn bw* cage and tested for sensitivity 303 days after the cage was started. The frequency of the three genotypes, *SD/SD*, SD/SD^+ and SD^+/SD^+ was 0.55, 0.42, and 0.03 respectively, giving a gene frequency for *SD* of 0.76. Fifteen *cn bw* chromosomes from this cage were tested for sensitivity, and of these, three showed very reduced k values in heterozygotes with *SD-5*; that is, k values less than 0.80. Thus insensitive alleles are recovered from populations originally consisting of only *SD* and sensitive SD^+ alleles. Since the total number of generations in this population cage was about 20 (supposing roughly 15 days for each generation), the observed frequency of insensitive SD^+ alleles is surprisingly high. Indeed, it seems too high to be

accounted for by selection alone. Thus, although there is certainly selection operating here, there are also probably other mechanisms causing the accumulation of insensitive alleles. In any event, it is true that insensitivity appears to be a concomitant of having *SD* in populations.

This raises a rather interesting point. Is it possible that a more sensitive test for *SD* in natural populations than a search for *SD* itself might be a survey for insensitive SD^+ alleles? This would be better for the reason that when enough insensitive alleles have accumulated in the population it could result in the elimination of *SD* altogether. Presumably, however, the insensitive alleles, being selectively indifferent once *SD* is eliminated from the population, would persist, and so such a survey could reveal, not only populations containing *SD*, but those populations which had possessed *SD* in the past.

Discussion

In a general way, it is clear that a locus, a chromosomal segment, or a whole chromosome, which exhibits meiotic drive, has an advantage for the reason it is present disproportionately often in the gametes contributing to each generation and, therefore, will tend to increase in frequency in a population.

The frequency of *SD* in natural poputions has not been as high as would be expected from the *k* values observed in the laboratory (Sandler, Hiraizumi, and Sandler, 1959). In the 1956 collection 6 *SD*-bearing second chromosomes were found among 183 tested chromosomes. In the 1958 collection there were 7 *SD*'s among a total of 301 chromosomes. These correspond to calculated allele frequencies of 3.3% and 2.3%. However, this is somewhat of an overestimate because the probability of detecting an *SD* allele from a heterozygous wild male is greater than 1/2. If the *k* value were 1, the values would be half as large, and this is a minimum estimate. The equilibrium value in the cage experiments was about 10%. Therefore there must be mechanisms suppressing the frequency of *SD* alleles in nature.

If the driven element has a selective fitness as high or higher than the alleles which are not driven, then it ought to become fixed in the population. Under these circumstances, the main effect of the phenomenon of meiotic drive would be to accelerate the rate of increase of this element in a manner analogous to periodic selection or orthoselection in bacteria (Atwood, *et al.*, 1951). If the driven element has a higher selective fitness than the other elements, this means an increase in the total fitness of the population. In this case, however, the maximum fitness toward which the population proceeds is the same irrespective of whether the most fit element is driven or not, but at any time prior to the attainment of maximum fitness, drive will cause a higher fitness.

Although this process may occasionally have happened in the course of evolution, it is likely to have been rare, for the obvious reason that most new mutants (or new genetic entities of any sort) wil have a lower fitness than the already existing ones and, therefore, the new instances of drive are usually selectively disadvantageous. Certainly it is true that all of the known cases of meiotic drive are associated with a reduction in fitness. Thus, the accumulation of the driven element will generally tend to lower the overall fitness of the population. This lowering of fitness may be quite extreme, but yet not sufficient to stop the accumulation of the driven element and thus may lead to extinction.

In nature, it is perhaps worth noting, the fitness of an equilibrium population must be determined in a very complicated way, and there may be conditions under which a driven element, which is itself detrimental, might cause an increase in the overall fitness of an equi-

librium population. The driven element, for example, might be linked with a beneficial gene and for a time at least, may cause an increase in the rate of accumulation of this beneficial linked gene, and hence initially increase the fitness of the population. Of course, eventually the linked gene and the driven element will become randomized with respect to each other and then, provided the driven element is still present, the population fitness will decline. However, if, in this process, some mechanism for overcoming the driven effect has been evolved, then the beneficial gene would have been incorporated into the population more rapidly than it could have otherwise.

An additional situation where a meiotic drive mechanism would be of advantage is where a balanced lethal situation already exists. In this case a mechanism that favors an excess of one chromosome in the gametes of one sex and the homologous chromosome in the other sex would decrease the proportion of lethal homozygotes. If a drive mechanism were to exist in one sex there would be strong selective pressure for such a mechanism acting on the homologous chromosome in the opposite sex.

From these considerations, we can conclude that by and large the population will suffer from having a meiotic drive mechanism, and unless there is some way in which the population can increase its own fitness even in the presence of a driven element, the population must either suffer a reduced fitness or find some mechanism to suppress or eliminate the effects of the driven element. Certain ways in which this might happen have been considered by Sandler and Novitski (1957).

In this connection, the segregation-distortion system is particularly interesting, because here we know at least one way by which the deleterious effect of *SD* was reduced. Namely, this population has accumulated insensitive SD^+ alleles which reduce the efficacy of the drive mechanism, and thus the equilibrium frequency of *SD*. Indeed, it may be that eventually insensitive alleles would become sufficiently widespread so as to cause elimination of *SD* entirely. We may recall that in Japanese populations in which no *SD* loci have been detected, no insensitive alleles were found.

We have seen that an insensitive allele will tend to replace a sensitive allele because of its greater frequency of transmission through *SD* heterozygotes. However, a non-allelic modifier of the *k* value would not have such an advantage. Thus, successful modifiers will tend to be allelic with the driven locus or very closely linked (if the cytogenetic mechanism permits).

It might be mentioned that if a mechanism of meiotic drive were to become homozygous in a population, the consequence would be that when this population is examined no apparent drive mechanism exists. It has occurred to us (and independently to others with whom we have discussed the problem) that an interesting screen for the detection of drive mechanisms would be to examine segregation ratios of hybrids carrying chromosomes from widely separated populations such as those from different continents.

Finally, meiotic drive has many kinetic properties in common with gametic, cytoplasmic, and asexual selection. Formally, meiotic drive is equivalent to gamete selection when the competition is only between gametes from the same individual. It is interesting to note that the rate of gene frequency change is exactly half as fast for meiotic drive as for selection between pooled gametes, as first shown by Haldane (1924).

Summary

An attempt has been made to demonstrate empirically three points: (1) That meiotic drive can be an operative evolutionary force. The demonstration of this is simply that we find, in natural popula-

tions of Drosophila, high frequencies of *SD* loci which are themselves associated with a reduced fitness. (2) That the quantitative predictions from considerations of what ought to happen when meiotic drive appears in a population are, roughly at least, true. The demonstration here is that the equilibrium frequencies and the rate of change in these frequencies in experimental cages of Drosophila containing *SD* are in rough agreement with quantitative prediction. And, finally, (3) to show at least one way in which a population, whose fitness is being reduced by the spread of detrimental driven loci may counteract this detrimental effect. In this instance, the result is accomplished by the accumulation of SD^+ alleles insensitive to the distorting action of *SD*. This accumulation is either by selection alone or possibly by selection and induction of insensitive alleles by a process analogous to (or identical with) the previously reported phenomenon of translocal modification. A mathematical analysis of a number of special cases of meiotic drive is included.

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INTROGRESSIVE HYBRIDIZATION BETWEEN TWO SOUTHEASTERN TREEFROGS [1]

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Natural interspecific hybridization in anuran amphibians is not rare, and a number of examples are recorded. There are, however, few conclusively proven instances of introgressive hybridization in the restricted sense of gene exchange between species. The best known case is that of the toads *Bufo woodhousei fowleri* and *Bufo terrestris americanus* (A. P. Blair, 1941; Volpe, 1952; and others). A related example is reported by A. P. Blair (1955) between *Bufo w. woodhousei* and *Bufo microscaphus,* and Wasserman (1957) provides evidence of introgression in the spadefoot toads *Scaphiopus hurteri* and *Scaphiopus couchi.* Most other presumed examples are based largely on simple identification of hybrids with no evidence for introgression, or belong to the borderline category of closely related contiguously allopatric forms that interbreed freely along a narrow zone of contact.

This paper deals with a clear case of interspecific gene exchange between the green treefrog, *Hyla cinerea,* and the barking treefrog, *Hyla gratiosa,* near Auburn, Alabama. Both species are widely distributed over the southeastern coastal plain. *H. gratiosa* ranges from North Carolina along the coastal plain (exclusive of southern Florida) to southeastern Louisiana, extending locally above the fall line, and onto the Cumberland Plateau. *H. cinerea* ranges from Maryland to south-central Texas, up the Mississippi Basin to southern Illinois, and into southern Florida. The two forms are, therefore, sympatric over the greater part of their ranges. The species are definitely related, and together with *Hyla andersoni,* may form a subgroup within the genus. Relationship is particularly evident in the nature of the breeding call (W. F. Blair, 1958a and b), but is also apparent in method of egg deposition and in certain larval features (Wright, 1932). Nevertheless, the two species are unusually distinctive in appearance. *H. gratiosa* is a large, stocky, heavily spotted form, while *H. cinerea* is smaller, more slender and unspotted, with a prominent light stripe on the side. The marked differences between the two species make them unusually favorable subjects for a study of this type.

Evidence of natural hybridization between the two species was first noted in material collected by the writer in 1957, and a detailed study of the populations was made in the spring and summer of 1958.

ENVIRONMENT

The study area. The locality in which the populations which form the basis for this study are found is situated on the piedmont plateau a few miles north of the fall line, approximately five miles north of Auburn, Alabama, just to the south of US 431 and just west of county road 147. The area is gently rolling and well drained, and has a considerable amount of tree cover, principally loblolly pine with a few hardwoods. The soil is a reddish brown sandy clay of granite or schist origin. Some 700 acres have been set aside here by the Agricultural Experiment Station of Auburn University for research on farm pond management. Scattered over the area are some 28 ponds

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(designated as the S series of ponds) which range in size from approximately one-half acre to 26 acres, although the greater number run from two to five acres. The margins of the ponds are planted in centipede grass, and weeds are kept in check by periodic mowing and spraying. The pond environment, therefore, is extremely artificial. Construction of these ponds began in 1945, with the greater number being completed by 1948. Dr. John Lawrence of the farm pond division at Auburn has informed the writer that there were no permanent ponds in this restricted area prior to 1945, and none of the present pond habitat could therefore have been in existence for longer than 12 years at the time the study was initiated.

According to the literature both *H. cinerea* and *H. gratiosa* reach their northern limit of general distribution at approximately this latitude in eastern Alabama, although the writer has collected *H. cinerea* ten miles farther to the north, and there is an isolated record for *H. gratiosa* some 60 miles to the north.

Comparative ecology. The two species have distinct environmental requirements. *H. cinerea* is an animal of moist situations, and it has been the writer's experience that it is rarely found far from permanent water, whereas *H. gratiosa* is more xeric adapted, and is characteristic of less moist and better drained habitats. Carr (1940) describes the habitat of *H. cinerea* in Florida as "swamp borders, seepage areas, and stream and lake margins," while that of *H. gratiosa* is "high pine, high hammock, and dry flatwoods, in the upper branches of longleaf and slash pine and of live oak." Similarly Wright (1949) states that *H. cinerea* occurs along the "swampy edges of stream courses; on the taller water plants in ditches or pools; on lily pads, trees, bushes, or vines not far from water." *H. gratiosa* is found in "trees of hammocks, pine barrens, and bays." *H. gratiosa* is recorded as burrowing in the soil during the winter or in hot dry weather, a habit not noted in *H. cinerea* and which is indicative of more terrestrial adaptations. Although both species occur throughout the study area, another series of ponds located only one mile to the south in an area of stream bottom soil and mesic woods support numerous *H. cinerea,* but *H. gratiosa* is apparently absent.

The two species differ markedly in general abundance. *H. gratiosa* is generally regarded as being uncommon and appears to be of more local and isolated occurrence throughout its range. Breeding aggregations are generally small (fewer than 20 to 25 individuals according to Carr), although larger congresses may occur (Goin, 1938). *H. cinerea,* on the other hand, is extremely common throughout most of its range, and calling males may form tremendous choruses, sometimes numbering in the hundreds or even thousands of individuals. The relative abundance of the two forms generally is reflected in their occurrence at the study locality, where *H. cinerea* is the more common by an overwhelming margin, and specimens can be collected in seemingly limitless numbers during peak breeding periods. *H. gratiosa* (or hybrid products between *H. gratiosa* and *H. cinerea*) is relatively rare, the breeding choruses small, and a much greater amount of effort was required to obtain material.

Population Analysis

Characters. Characters used in the population analysis are as follows:

(a). Snout-urostyle length. This measment is used as an expression of body length, and is more accurate (although slightly lower) than the more conventional snout-vent measurement.

(b). Head width. This measurement expressed relative to snout-urostyle length has a low variability and is very useful for separation of the two species. This and other measurements expressed as body proportions are subject to some allometric variation, but the differences are of no consequence in the size range of the sex-

ually mature individuals used in this study.

(c). Height of spade (inner metatarsal tubercle). This measurement, expressed as a percentage of snout-urostyle length, has also proven to be a very reliable character.

(d). Tibio-fibula length. This measurement has also been expressed as a percentage of snout-urostyle length. Although useful, this character is subject to more variation than the preceding ones.

(e). Lateral stripe. The degree of development of a light stripe on the side has been measured on the basis of a scoring system ranging from zero to six, based on length of the stripe, irregularity, and definition.

(f). Dorsal spotting. Although the presence or absence of dark dorsal spots is one of the more obvious differences between the two species, it has proven to be one of the most difficult to measure objectively as the spots may vary independently as to number, size, shape, and intensity. For this reason dorsal spotting has been scored only as completely absent, well developed as in normal *H. gratiosa* from nonintrogressing populations, or present but definitely reduced as compared with the normal range of *H. gratiosa*. In some unusually dark specimens (many of the preserved hybrids fell in this category) the dorsal spots were difficult to observe, but could be detected under water in strong light. Occasional bright green *H. gratiosa* occur in which the dorsal spots are obscured. Although several examples used in the study fell in this category, spotting was still visible upon the lateral and posterior parts of the dorsum.

Differences in other characters could be observed between the two species, but were found to be less reliable or difficult to measure, and were not used in the analysis. The skin surface of *H. gratiosa* is more granular than that of *H. cinerea*, but the difference defies objective measurement. The degree of development of a light line on the posterior surface of the tibio-fibula and tarsal element is correlated with development of the lateral stripe, but is more variable. The intensity of throat pigmentation in *H. gratiosa* males is generally greater than in *H. cinerea* males, but again is difficult to measure.

All body measurements used in the analysis were made with the aid of vernier callipers, with the exception of spade height which was measured with an ocular micrometer.

Analysis by pictorialized scatter diagram. In this analysis (figs. 1 and 2) head width is plotted against snout-urostyle length. Well developed dorsal spotting is indicated by a dark circle, reduced spotting by a dark-centered circle, and complete absence of dark dorsal spots by a light circle. Height of spade as a percentage of snout-urostyle length is indicated by the length of the horizontal bar to the right of the circle, expressing the excess of 1.0% in a range of 1.0–3.3%. Tibio-fibula length as a percentage of snout-urostyle length is indicated by the length of the oblique bar on the upper left, expressing the excess of 44.0% in a range of 44.0–57.8%. Degree of development of the lateral stripe is indicated by the length of the vertical bar on the lower side of the circle, on a scale from zero (completely absent) to six (maximal development).

In order to establish the degree of development of the various characters in populations free from the effects of introgression a comparison has been made between a series of *H. gratiosa* from Alachua County, Florida, and *H. cinerea* from several localities in Lee County, Alabama, where *H. gratiosa* appears to be absent (fig. 1). The diagram indicates that the two species are different on the basis of every character with no overlap except in snout-urostyle length. *H. gratiosa* has a relatively wider head and larger spade, averages larger in size, and always possesses dark dorsal spots. *H. cinerea* has a relatively longer tibio-fibula and a much better developed lateral stripe, and consistently lacks dark dorsal spotting.

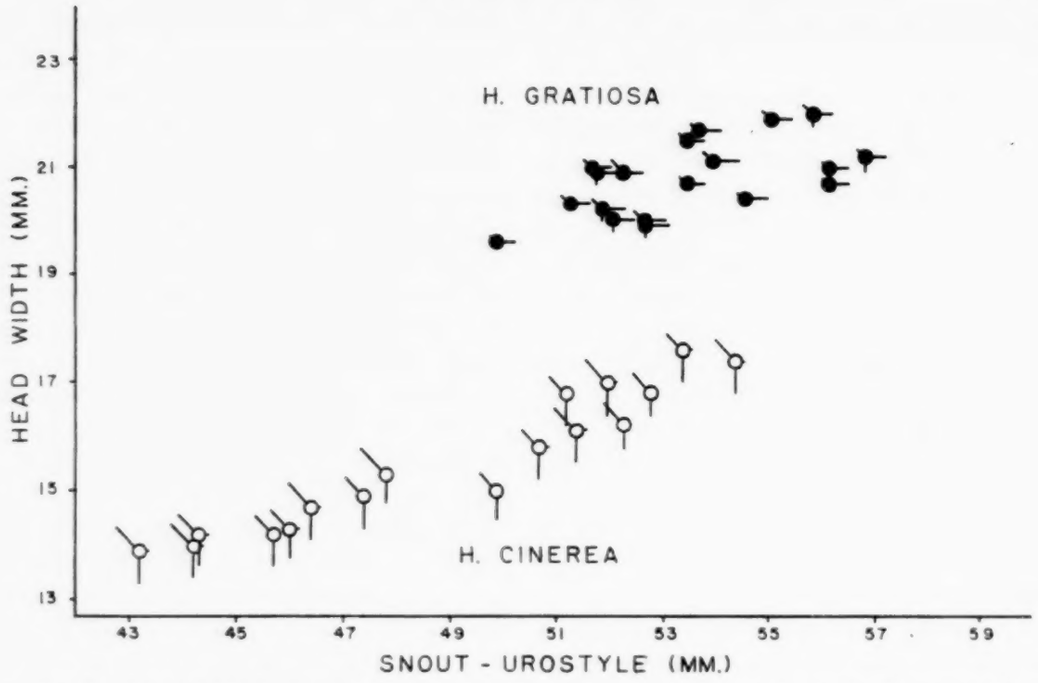

FIG. 1. Pictorialized scatter diagram showing the characteristics of genetically unmodified *H. gratiosa* from Alachua County, Florida, and *H. cinerea* from Lee County, Alabama. Dark circles represent dorsal spotting, light circles no dorsal spotting; relative height of spade is indicated by the horizontal bar to the right of the circle; relative length of tibiofibula is indicated by the length of the oblique bar on the upper left; development of a lateral stripe is indicated by the length of the vertical bar below the circle. More detailed information is given in the text.

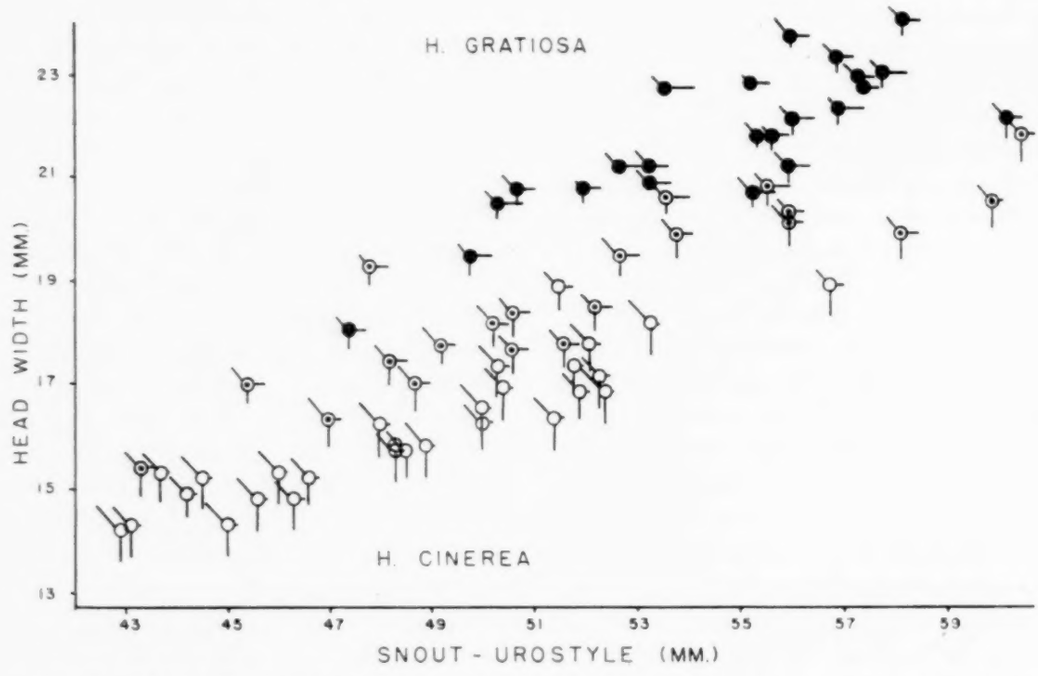

FIG. 2. Pictorialized scatter diagram showing the characteristics of a series of specimens from introgressing populations of *H. gratiosa* and *H. cinerea* from north of Auburn, Lee County, Alabama. A dark-centered circle indicates reduced dorsal spotting; other elements of the symbols are the same as in figure 1.

The same characters have been plotted for specimens from the study area north of Auburn (fig. 2). All *H. gratiosa* and presumed hybrids and backcross products collected at the area through the summer of 1958 have been included, although the number of typical *H. cinerea* available was so large that only a random sample has been analyzed. It is evident that extensive hybridization is taking place. Most specimens which fall in an intermediate zone with respect to head width also appear intermediate in other characters as well, as might be expected when dealing with polygenic characters. Comparison with the control series on figure 1 indicates backcrossing with both parent species, particularly with *H. gratiosa*.

Analysis by hybrid index. In an effort to obtain a clearer indication of the extent of hybridization and the degree to which each parent species is maintaining its identity each specimen plotted on the two scatter diagrams has been individually scored for each of the six different characters measured, and these scores have been added together to give a hybrid index for the specimen. The scoring system is as follows: Head width and height of spade, both expressed as a percentage of snout-urostyle length, are objective measurements with a high degree of consistency and each has therefore been assigned the relatively high possible score of 12. In the case of head width this is the equivalent of the excess of 30.0% in a range to 42.2%, i.e., 30.0% = 0, 42.2% = 12. In the case of the height of the spade the score is the equivalent of the excess of 1.0% in a range to 3.3%. Tibio-fibula length expressed as a percentage of snout-urostyle length is an objectively measured character but is less consistent than the preceding. For this reason it has been assigned a possible score of ten, and is the equivalent of the excess of 44.0% in a range to 57.8%. The degree of development of the lateral stripe is a striking difference between the two species, but is less objectively measured. For this reason it has been assigned a possible score of six. The development of dorsal spotting is even more difficult to measure, and has therefore been assigned a possible score of only four. The considerable overlap in snout-urostyle length indicates that this character should not be weighed too heavily. It has been assigned a possible score of four, and is the equivalent of the excess of 42.9 mm in a range to 60.6 mm. Scores for head width, height of spade, degree of spotting, and size are higher in *H. gratiosa* than in *H. cinerea* and have been assigned positive values. Scores for tibio-fibula length and lateral stripe are higher in *H. cinerea* than in *H. gratiosa,* and have been assigned negative values.

The frequency distributions of the hybrid indices obtained for the control series of *H. gratiosa* and *H. cinerea* and for specimens from the study area are presented in the form of histograms in figure 3. The control series are remarkably distinct, a condition already demonstrated by the scatter diagram analysis, and extensive hybridization is again clearly evident between the populations north of Auburn. It may be seen that the

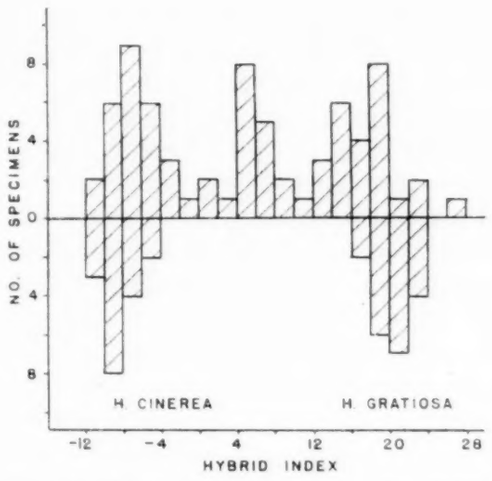

FIG. 3. Histograms indicating the distribution of hybrid index values of specimens from introgressing and control populations of *H. cinerea* and *H. gratiosa*. Upper histogram: introgressing populations from north of Auburn, Alabama. Inverted histogram: genetically unmodified *H. gratiosa* from Alachua County, Florida, and *H. cinerea* from Lee County, Alabama.

H. cinerea and *H. gratiosa*-like specimens form fairly distinct groups at opposite ends of the distribution, indicating that neither species has lost its identity as a separate breeding population. However, backcrossing to both parent species is indicated by a skewing of the distribution of each toward the values of the other. This backcrossing appears to have been particularly extensive in the case of *H. gratiosa,* where it seems that the greater part of the population is made up of individuals with at least some elements of the *H. cinerea* genotype. The bulk of the *H. cinerea* seems largely unmodified, although a few specimens show clearly the effects of backcrossing. In this connection it should be recalled that only a random sample of numerous typical *H. cinerea* from the study area were analyzed, whereas all presumed hybrids, backcross hybrids, and *H. gratiosa* were included in the analysis. The relative number of typical *H. cinerea* in the population, therefore, may be assumed to be considerably greater than is indicated by the histogram. The existence of a distinct hybrid element (the central group in the histogram) approaching in numbers the *gratiosa*-like segment is particularly interesting. On the basis of morphology it is clear that these specimens cannot be the result of backcrosses, and therefore must be either F_1 hybrids or progeny of crosses between hybrids. It is more reasonable to suppose that most of these specimens, at least, fall in the latter category. It seems highly unlikely that reproductive isolation between two such broadly sympatric species would be suddenly relaxed to such an extreme degree, or that cross-mating could take place so freely as to produce such numbers of F_1 hybrids without a rapid and complete loss of the identity of the species. The criticism might be raised that the formation of an intermediate peak on the histogram by hybrid types is an artifact of selective collecting for hybrids. While there was a certain bias in collecting, this bias was directed about equally toward hybrids, backcross products, and *H. gratiosa,* whereas typical *H. cinerea,* being extremely abundant, were collected only in haphazard fashion and only a random sample of those collected was analyzed. It is unlikely, therefore, that selectivity in collecting resulted in any exaggeration of the distinctiveness of the hybrid group, although the distinctiveness of this group as compared with *H. cinerea* was probably minimized as apparent backcross products in the direction of *H. cinerea* were collected more diligently than typical *H. cinerea.*

Reproductive Isolating Mechanisms

Before attempting to identify the factors which have led to the breakdown of the species barriers, it is necessary to consider the relative importance of the various types of reproductive isolating mechanisms which may normally act to maintain the identity of the two forms. A number of possible mechanisms must be taken into account.

Hybrid viability and fertility. A number of crosses were made with the object of establishing the degree of interfertility in the interspecific cross and level of fertility in the hybrid. All individuals utilized in the experiments with the exception of two *H. gratiosa* males and three *H. cinerea* males were collected at the study area, and all were either clearly *H. cinerea, H. gratiosa,* or definite hybrids in morphological characteristics. All crosses were made artificially by stripping eggs from the female directly into a sperm suspension prepared by macerating testes in five cc. of pond water. Results of these experiments are summarized in table 1. The percentage of normal development to the larval stage is expressed as the percentage of those eggs which underwent cleavage. This is less misleading than expressing normal development in terms of total number of eggs because in a few cases involving *H. cinerea* males the level of fertilization (as indicated by rotation and cleavage) was markedly reduced. This reduction

TABLE 1. *Results of crosses involving* H. cinerea, H. gratiosa, *and natural hybrids between the two species*

Cross No.	Females	Males	Egg No.	Cleavage	Blastula	Neurula	Tail fin circulation	Normal larvae	% Normal devel. in cleaving eggs
1	*cinerea* (A)	*cinerea* (a)	105	56	56	56	55	55	98.2
2	*cinerea* (A)	*gratiosa* (a)	137	133	133	132	131	131	98.5
3	*cinerea* (B)	*cinerea* (b)	154	145	144	143	141	141	97.2
4	*cinerea* (B)	*gratiosa* (b)	113	111	111	110	108	108	98.0
5	*cinerea* (B)	Hybrid (a)	133	129	128	126	126	124	96.1
6	*cinerea* (B)	Hybrid (b)	110	105	140	103	103	102	97.1
7	*cinerea* (C)	*cinerea* (c)	91	86	85	83	83	83	96.5
8	*cinerea* (C)	*gratiosa* (b)	114	114	113	113	113	113	99.1
9	*cinerea* (C)	Hybrid (a)	127	125	125	125	124	124	99.2
10	*cinerea* (C)	Hybrid (b)	141	141	141	141	140	140	99.3
11	*gratiosa* (A)	*gratiosa* (c)	118	110	110	104	103	102	92.7
12	*gratiosa* (A)	*cinerea* (d)	243	238	238	236	221	217	90.8
13	*gratiosa* (A)	*cinerea* (e)	107	57	57	57	55	55	96.5
14	*gratiosa* (A)	Hybrid (c)	112	110	110	108	104	104	94.5
15	*gratiosa* (B)	*gratiosa* (d)	143	131	140	140	139	125	88.7
16	*gratiosa* (B)	*cinerea* (f)	115	47	46	46	40	35	74.5
17	*gratiosa* (B)	*cinerea* (g)	122	106	104	104	91	83	78.3
18	*gratiosa* (C)	*gratios* (e)	61	58	58	58	58	56	96.6
19	*gratiosa* (C)	*cinerea* (h)	89	75	75	75	74	73	97.4
20	*gratiosa* (C)	Hybrid (d)	77	75	75	73	70	70	93.3
21	Hybrid (A)	*cinerea* (i)	92	90	90	80	67	65	72.2
22	Hybrid (A)	Hybrid (d)	75	74	74	65	57	55	74.3

apparently was due to partial sterility of the male rather than genetic incompatibility. While it was evident in two of the interspecific crosses (Nos. 13 and 16), it also showed up in two of the controls (No. 1, and a duplicate control not included in the table).

The *H. cinerea* female × *H. gratiosa* male cross was made three times, involving three *H. cinerea* females and two *H. gratiosa* males (Nos. 2, 4, and 8, table 1). In all cases the level of normal development in the hybrid combinations was at least as high as in the controls. The reciprocal cross, *H. gratiosa* female × *H. cinerea* male, was made five times, involving three females and four males. In two of these crosses (Nos. 16, 17) normal development in the hybrid embryos fell slightly below the control, but levels of normal development in the remaining crosses (Nos. 12, 13, 19) agreed closely with the controls. The fertility of two natural hybrids was tested in backcrosses with two *H. cinerea* females respectively (Nos. 5, 6, 9, 10). In all cases the level of normal development was comparable to that of the control. Two other natural hybrids were tested in backcrosses with *H. gratiosa* females (Nos. 14, 20), and again the level of normal development was high and agreed closely with the controls. A single hybrid female was tested in a backcross to a *H. cinerea* male and with another hybrid (Nos. 21 and 22 respectively). The level of normal development was slightly reduced in both cases (72.2 and 74.3% respectively), possibly indicating some degree of breakdown in crosses involving the hybrid female, but the results must be accepted with considerable reservation as no control was possible with regard to the female. Larvae from the various crosses were carried through metamorphosis without any evidence of abnormalities.

To summarize the results of these experiments, no clear evidence of a reduction in the level of fertilization or normal development was obtained in any crosses involving *H. cinerea* and *H. gratiosa* or hybrids between these two species, although in a few cases, particularly two involving a hybrid female, there were indications of a slight reduction in normal development. The objection might be raised that since most of the specimens used in these crosses came from populations undergoing natural hybridation, the levels of crossability and hybrid fertility may be abnormally high due to differential gene survival in the popula-

tions in favor of those genes promoting interfertility. However, the large amount of morphological difference between the species makes the selection of a genetically "pure" specimen a fairly reliable procedure, and in the case of the population of *H. cinerea,* at least, the effects of backcrossing have not been extensive. The objection has more validity as applied to natural hybrids. Although hybrids used in the experiments appeared morphologically comparable to what might be expected in F_1s, it is difficult if not impossible to distinguish between an F_1 hybrid and many progeny of hybrids, and it is possible that hereditary material promoting interfertility in some of these examples had been subjected to selection through a number of generations. On the other hand, in six crosses made with natural hybrids there was no evidence whatever of reduced fertility, and it is probable that if a high level of sterility or developmental incompatibility did exist in crosses involving hybrids, it would have been evident to at least some degree in these crosses. It must be concluded that the barriers of hybrid inviability and sterility between these two species are both at least low, if not absent.

Temporal isolation. Although a difference in breeding season has been shown to be of importance as an isolating mechanism in some Anura, it appears to be of no significance in the present case. Carr (1940) gives the breeding season in Florida as March 3 to August 14 for *H. gratiosa* and March 8 to the middle of August for *H. cinerea.* Clasping pairs of both species have been recorded in the Auburn area as early as April 21 and as late as August 15, with a period of peak breeding activity for both species from approximately late April to the middle of June. The environmental stimuli that induce breeding activity within the breeding season do not appear to differ significantly. Rain is not necessary in either form for breeding, although warm rains generally lead to the greatest activity, and calling activity is reduced in both species after any prolonged period without rain. Humid overcast conditions appear to produce the most activity in both forms. Both species may call at temperatures ranging down to at least 16 degrees C., although the optimal range for both appears to be in excess of 20 degrees C. There was some indication that *H. gratiosa* called more actively than *H. cinerea* on some cool spring nights, but this may have been due to the fact that *H. gratiosa* calls from the water and is therefore less susceptible to a decrease in evening temperature. In spite of the foregoing similarities there does appear to be at least some temporal isolation between the two species due to a random factor in formation of breeding aggregations, particularly outside of times of peak breeding activity. Although choruses of both species occur throughout the study area at one time or another, no pond is consistently utilized by either species throughout the breeding season, and their formation at any one pond seems to be more or less on the basis of chance. Thus a certain pond where *H. gratiosa* is calling vigorously at one time might, a week or so later, show no activity whatsoever, while a distant pond where no *H. gratiosa* has been heard previously might have an active chorus. As the two species appear to form calling aggregations independently, this factor of chance leads to some independent breeding at any one pond.

Habitat isolation. Although the two species appear to have distinct ecological requirements, these can be of importance in reproductive isolation only to the extent that they determine differences in the breeding site. In actual practice, although *H. cinerea* breeds in a variety of situations which are never utilized by *H. gratiosa,* ponds to which *H. gratiosa* may repair for breeding frequently support populations of *H. cinerea,* and it is the writers' opinion based on field experience in Florida as well as in Alabama that *H. gratiosa* breeds as often as not in ponds also frequented by *H. cinerea.* Ecological overlap with respect to breeding site

therefore, is not unusual, and does not necessarily lead to hybridization. The series of *H. gratiosa* from Florida used for comparison in the population analysis was collected in mixed chorus with several other species of *Hyla,* including numerous *H. cinerea,* and yet shows no evidence of introgression.

Ethological isolation. In view of the manifestly incomplete nature of the isolating factors considered up to this point, it seems probable that the most critical reproductive isolating mechanisms fall in this last category. These factors would determine selective mating within each species in mixed breeding aggregations. Observations of clasping pairs indicate that even under the artificial conditions of the study locality the clasping pattern is far from random, in spite of the fact that numerous natural hybrids attest to breakdown of reproductive isolation. At no time during the study was the writer fortunate enough to find an amplexing pair in which one member was clearly a *H. cinerea* and the other a *H. gratiosa,* although many clasping pairs of both species were observed. There was even some evidence based on direct observation of selective mating between hybrids themselves. In two instances hybrid males were found clasping hybrid females. One pair was found at a pond where numerous *H. cinerea,* a few *H. gratiosa,* and a few hybrids were calling. The other pair was collected at a pond with a heavy chorus of *H. cinerea* (numbering in the hundreds of individuals) among which the calls of two hybrids could be distinguished. The relative scarcity of hybrids in both of these breeding aggregations indicates very strongly that the amplexing hybrid pairs cannot be attributed to chance, and must be the result of selective location of a hybrid by a hybrid. One instance was found of a specimen of hybrid nature in amplexus with one of the parent species, in this case a hybrid male and a *H. cinerea* female. The hybrid, however, was recorded as tending toward *H. cinerea* in general appearance, and may have been the product of backcrossing in the direction of *H. cinerea.* In several other instances clasping pairs were found in which one member showed at least a trace of foreign hereditary material.

Of the various factors which function in ethological isolation, possibly the most important is selective response by the female to the breeding call of the male. The fundamental role of call in location of the breeding site and attraction of the female in many anurans cannot be doubted, and it is reasonable to suppose that call differences between sympatric species are often effective in reproductive isolation. Support for this assumption has recently been furnished by W. F. Blair (1959) and Martof (1959), both of whom obtained evidence of selective response on the part of females of certain species to the recorded calls of their own species. W. F. Blair (1958a and b) has analyzed the calls of *H. cinerea* and *H. gratiosa* by means of sound spectrograms. He indicates that the calls of the two species are of the same basic type, with the chief difference being the fact that in *H. cinerea* the second harmonic of the initial part of the call becomes the fundamental of the body of the call, while in *H. gratiosa* the third harmonic does so. Inspection of the figures of the spectrograms indicates that differences in emphasized frequencies are also of considerable importance. In any event, the calls of the two species are readily distinguishable to the human ear. The call of *H. gratiosa* heard at a distance is not unlike the deep bark of a dog (hence the common name barking frog) while the call of *H. cinerea* (to the writer's ear at least) sounds somewhat higher pitched and more nasal with a quacking quality. The call of *H. gratiosa* carries a greater distance, and is more audible in a mixed chorus. Calls of hybrids are intermediate in sound, and with a little experience can be readily identified. Hybrid vocalization seems to be generally normal, although in two instances specimens which

appeared to be having difficulty in producing a call proved upon capture to be hybrids. It is possible that the tendency already noted toward mating between hybrids is due to a selective response on the part of a hybrid female to the intermediate call.

Another ethological difference between the two species which may be of considerable importance is the calling position of the male. The male *H. cinerea* never gives the mating call from the water. Calling individuals are located near the pond margin on the ground, on grasses or sedges, on bushes, or on trunks and branches of trees at a fairly low height. *H. gratiosa,* on the other hand, typically calls while floating in an inflated condition in open water. Occasional specimens call while sitting in the water at the edge of the pond, but it may be that in such cases the animal has only recently arrived at the pond. In a few instances specimens were recorded calling from emergent logs or branches in open water, but this was atypical. If we may assume a corresponding difference in the behavior of the female in the type of situation she will enter in seeking out the male, the result would be at least partial reproductive isolation. It seems unlikely that a *H. cinerea* female would enter the water and swim out to a *H. gratiosa* male, although position isolation in the reciprocal combination would probably be much less effective. Interestingly enough, no hybrid males were ever recorded as calling from a floating position, but rather occupied positions similar to those of *H. cinerea* males. On several occasions hybrids were observed calling from pilings in the open water. This would seem to indicate that hybrids will enter the water more readily than *H. cinerea,* although they cannot call from the floating position.

Other ethological factors may be of some importance, but are more difficult to evaluate. There may be some tactile discrimination by the male with regard to differences in skin texture, size, or general proportions, but the writer has observed freshly collected specimens to cross clasp in aquaria upon a number of occasions, and it seems unlikely that this type of discrimination is of great significance. It is possible, however, that tactile influences are more effective under natural conditions and in specimens of a lower level of sexual excitation, and the larger average body size and relatively greater girth of *H. gratiosa* undoubtedly presents some mechanical difficulties in cross clasping between larger *H. gratiosa* and smaller *H. cinerea.* Visual discrimination based on pattern or general appearance is also a possibility. However, the species breed at night, particularly under overcast conditions, and although close range visual location undoubtedly takes place, detailed visual discrimination under such conditions seems unlikely. The possibility of olfactory discrimination is discounted but not eliminated. *H. gratiosa* does have a noticeably stronger odor and secretes a more copious supply of mucus when handled.

Basis for breakdown in reproductive isolation. The explanation for the failure of reproductive isolating mechanisms at the study locality undoubtedly lies in the altered nature of the environment. It may be assumed that before construction of the ponds the number of *H. cinerea* in the area was low because of the scarcity of marginal aquatic habitats in an upland situation, although the better adapted *H. gratiosa* was probably present in at least moderate numbers. With construction of the series of ponds an unusually large population of *H. cinerea* rapidly became established, although *H. gratiosa* failed to show a comparable increase. Just why there was this differential increase is uncertain, but it may have been due to the fact that a large amount of habitat favorable for *H. cinerea* was created as a direct result of pond construction, whereas the suitability of the area for *H. gratiosa* was not changed except for the addition of more breeding sites. In any event, the result is that congresses of *H. gratiosa* now often breed in company with over-

whelming numbers of *H. cinerea*. As already pointed out, the mere fact that the two species overlap at a breeding site does not necessarily result in hybridization, but the unusual abundance and predominance of one species in this case may be a distinctive feature. Another factor of possibly even greater importance is the artificial nature of the ponds themselves. As already indicated, the margins of the ponds are kept relatively clear of high grass and other vegetation through mowing and spraying, and there is an abnormally small amount of cover for the frogs. Both of these factors, large numbers of *H. cinerea* and an absence of concealing vegetation, increase the chance of close range visual location or even body contact between the species, thereby reducing the presumed effectiveness of selective location of the male by the female through call discrimination. The writer has observed *H. cinerea* males upon several occasions to leap upon, and in one case actually pursue other *H. cinerea* which moved in their immediate vicinity, and it is easy to see how a *H. gratiosa* female moving to the breeding pond may pass too close to a *H. cinerea* male and may be clasped by that male. Occurrence of the reciprocal combination seems less likely because of the aquatic calling position of the male *H. gratiosa*, although it is not impossible that one of the few *H. gratiosa* males calling from the pond margin might intercept a *H. cinerea* female.

Discussion

The morphological analysis by way of pictorialized scatter diagram and hybrid index of populations of the treefrogs *Hyla gratiosa* and *Hyla cinerea* from an area north of Auburn, Alabama, demonstrates conclusively the occurrence of extensive introgressive hybridization between the two species. It seems clear that of the two parent populations, *H. gratiosa* is the more modified by the effects of introgression. The explanation for this condition is not clear. Although the population of *H. cinerea* greatly outnumbers that of *H. gratiosa*, if backcrossing by hybrids is random, it would be expected that backcrossing to each parental type would take place with a frequency proportional to the numbers of each species, and both would therefore become modified to an equal degree. Hybrids appear to be equally fertile in experimental backcrosses with either parental species, and while it is conceivable that some other reproductive factor or factors isolate hybrids more effectively from *H. cinerea* than from *H. gratiosa*, this seems unlikely. A more reasonable explanation is that there is a more extensive migration of unmodified *H. cinerea* into the study area. The existence of a large population of *H. cinerea* in the vicinity of a series of experimental ponds only one mile to the south has already been noted, and large choruses of this species were also recorded at a farm pond approximately 0.8 miles to the east of the study area. These and other populations of *H. cinerea* may be close enough to the study area to allow for a considerable immigration of *H. cinerea*.

One product of the analysis was the demonstration of an unexpectedly large number of hybrid individuals which could be explained on the basis of their characters only as F_1 hybrids or progeny of matings between hybrids. As already indicated, it seems unlikely that if reproductive barriers had been relaxed to the extent necessary to account for these individuals as F_1 hybrids, that both parental forms could have maintained their identity. In addition, observations on clasping pairs did not indicate that cross mating is occurring with any great frequency. Both considerations lead to the conclusion that the intermediate group is represented for the most part by progeny of matings between hybrids. This being so, then there must be some degree of selective mating between hybrids because of the fact that under conditions of random mating hybrids would be much more likely to pair with one of the numerous

individuals of either parental species rather than with one of the (at least initially) rare hybrids, and offspring of matings between hybrids would be expected to be uncommon. The presence of a fairly large hybrid group (approaching *H. gratiosa* in population size) would therefore seem to indicate selective mating between hybrids, a conclusion which receives considerable support from the observation of two hybrid amplexing pairs under conditions which made chance contact of hybrids with hybrids highly improbable. It seems reasonable to conclude, therefore, that hybrids tend to mate with hybrids, and form a third population which is at least partially isolated reproductively from both of the parent species. This should not be taken to imply that the case under study is an example of sympatric speciation. Granted that some reproductive isolation of hybrids exists, the considerable degree of backcrossing to the parental species, particularly obvious in the case of *H. gratiosa,* clearly indicates that this reproductive isolation is not at a high level, and the present situation at best may be only a transitory one. Nevertheless, it does suggest a mechanism by which sympatric speciation might conceivably occur. If an ethological factor (or factors) which functions in reproductive isolation between two interfertile species is so well defined that hybrids tend to select hybrids in which sensory cues associated with this factor are of intermediate development, and further, if a sufficient number of genes without close linkage are involved so that variation due to recombination in offspring of hybrids is not great, then the origin of populations more or less isolated reproductively from the parent types could occur through hybridization. It seems more likely that this would happen under conditions of limited as opposed to extensive hybridization, as the level of reproductive isolation of the hybrids would be expected to be correlated (although perhaps at a lower level) with the level of isolation between the parental species. If the initial hybridization was due to a local and temporary relaxation of complementary isolating factors, it is not inconceivable that with restoration of more natural conditions the hybrid population could persist and occupy a new or intermediate adaptive zone as a new species. Such a hypothesis is attractive because it avoids the major stumbling block to most theories of the origin of new species through hybridization; the absence of reproductive isolating mechanisms (aside from sterility barriers arising through amphiploidy) which would be immediately effective in preventing free interbreeding with either of the parental species.

Although few of the reproductive isolating mechanisms which operate between *H. gratiosa* and *H. cinerea* are adequately understood, it is clear that reproductive isolation is brought about through the combined action of a number of different mechanisms which vary widely in effectiveness. The barriers formed by hybrid inviability and sterility appear to be very low, and temporal isolation is only slightly effective. Habitat isolation is doubtless of some value, but the species breed together in many areas without breakdown of the species barriers. It is probable that the most critical isolating factors are those which determine selective location between individuals at the breeding site. Call discrimination by the female is probably of outstanding importance in this respect, although isolation through calling position is undoubtedly important in the *H. gratiosa* male-*H. cinerea* female combination, and other ethological factors such as tactile discrimination may be operative to some extent at close range.

As pointed out by Anderson (1949), Dobzhansky (1951), and others, natural hybridization is frequently the result of a disturbance of the environment through the activities of man. The present case clearly falls in this category. The factors involved appear to be a disproportionately large population of *H. cinerea* created as a direct result of construction

of artificial ponds, and an absence of vegetational cover in the immediate vicinity of the ponds. Both factors act in a complementary fashion to greatly increase the probability of chance contact between individuals and thereby lower the efficiency of mechanisms which favor the selective location by females of males of their own species. It may be assumed that even under more natural conditions there is always at least a small chance of breakdown of reproductive isolation wherever the two species breed together. Bogert (1958) has identified a hybrid from Highlands County, Florida, and Smith and List (1955) have described as a presumed *H. cinerea* a specimen from Harrison County, Mississippi, with pattern characteristics which strongly indicate that the example is actually a hybrid. It is not improbable that occasional hybridization between the two species is a phenomenon of long standing.

Summary

Morphological analysis of populations of the green treefrog, *Hyla cinerea*, and the barking treefrog, *Hyla gratiosa*, from an area of experimental farm ponds near Auburn, Alabama, has revealed considerable introgressive hybridization between the two species. Numerous natural hybrids in the area appear to form a breeding population partially isolated reproductively from either parent species. Experimental crosses between the two species and with natural hybrids indicate high levels of hybrid viability and fertility. The cause of the breakdown of the reproductive barriers between the species undoubtedly lies with the artificial nature of the breeding ponds. The lack of vegetational cover around the margins of the ponds, together with the presence of a disproportionately large population of *H. cinerea* which did not exist in the area before construction of the ponds, has greatly increased the probability of chance contact between the species at the breeding site, thereby impairing the effectiveness of reproductive isolating mechanisms favoring selective location of members of the same species.

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THE EVOLUTIONARY DYNAMICS OF COMPLEX POLYMORPHISMS[1,2,3]

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The theory of balanced polymorphism which has been elaborated by population geneticists, notably Sewall Wright and R. A. Fisher, has in the main been concerned with the effects of single loci. As theory generally goes apace with experiment, this accent on single locus polymorphisms has been due to the plethora of observational evidence relating to simple cases. It is sufficient to note the vast effort made by Dobzhansky and his co-workers in their elucidation of the inversion polymorphism of the third chromosome of *Drosophila pseudoobscura*.

In recent years, however, a few cases have come to light of polymorphisms involving more than one Mendelian unit. Among these are the inversions on different chromosomes found in *D. robusta* studied by Levitan (1955 and 1958), the shell color of *Cepaea nemoralis* reported by Lamotte (1951) and by Cain and Sheppard (1952), the complex mimicry pattern in certain butterflies (Sheppard, 1959) and the inversions in two chromosomes of the grasshopper *Moraba scurra* analyzed by White (1957) and Lewontin and White (1960).

The study of effects of natural selection on single locus polymorphisms must take into account only inter-allelic effects such as additivity and dominance. In multi-locus polymorphisms, however, two new factors add more complexity. They are recombination between loci and interactions between the loci in determining fitnesses of genotypes (*epistasis* in the broad sense).

Some theoretical work has been done on selection in multi-locus systems, notably the paper of Kimura (1956) on a special case of epistasis and heterosis. Kimura's equations (p. 279) are, however, quite general in their applicability and are completely analogous to our equations 12a–12d although arrived at from a different point of view.

It is our purpose in this article to present the basic theoretical considerations for the analysis of multiple polymorphisms, by examining in some detail the evolutionary dynamics of two-locus systems. It is believed, in view of the results to be presented, that no great gain in understanding would be made by an extremely unwieldy analysis of more complex systems.

LINKAGE EQUILIBRIUM AND DISEQUILIBRIUM

Essential to a discussion of the two-locus problem is the concept of *linkage equilibrium*. This problem has been dealt with by Geiringer (1944) and can be summarized as follows: Suppose that there are two segregating loci, A–a and B–b. There will then be four possible gametic types: AB, Ab, aB and ab. Let the frequencies of these four gametic types be g_{11}, g_{10}, g_{01}, g_{00}, respectively. These frequencies satisfy the following

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relationship:

$g_{11}+g_{10}=p$, the frequency of allele A,
$g_{01}+g_{00}=(1-p)$, the frequency of allele a,
$g_{11}+g_{01}=r$, the frequency of allele B,
$g_{10}+g_{00}=(1-r)$, the frequency of allele b.

Linkage equilibrium will be defined by the condition that each gametic frequency is the product of the appropriate gene frequencies. That is, at linkage equilibrium

$$\hat{g}_{11}=pr, \qquad \hat{g}_{10}=p(1-r),$$
$$\hat{g}_{01}=(1-p)r, \quad \hat{g}_{00}=(1-p)(1-r). \tag{1}$$

It can be shown that at a non-equilibrium condition the gametes will have the frequencies

$$\begin{aligned} g_{11} &= \hat{g}_{11} + D, \quad g_{10} = \hat{g}_{10} - D, \\ g_{01} &= \hat{g}_{01} - D, \quad g_{00} = \hat{g}_{00} + D, \end{aligned} \tag{2}$$

where $D = g_{11}g_{00} - g_{10}g_{01}$. The equations in (2) imply that any time the gametic frequencies will deviate from the equilibrium frequencies by an amount D which is the product of the coupling gametic frequencies minus that of the repulsion gametic frequencies. D, thus defined, may be considered as a measure of *linkage disequilibrium*. Obviously, at equilibrium

$$\hat{D}=\hat{g}_{11}\hat{g}_{00}-\hat{g}_{10}\hat{g}_{01}$$
$$=pr(1-p)(1-r)-p(1-r)r(1-p)=0.$$

Finally it should be noted that in the absence of any evolutionary pressure such as selection, the value of D will approach zero with succeeding generations. The relation between the initial value, D_0, and the value in the n-th generation through random mating is

$$D_n = (1 - R)^n D_0 \tag{3}$$

where R is the recombination frequency between the loci. It is important to stress that the relation (3) is true only when no other evolutionary forces are assumed, because, as will be shown, natural selection may upset the approach to eventual linkage equilibrium.

Populations in Linkage Equilibrium

The approach used in this paper for investigating the interactions of recombination and gene effects is to first assume, incorrectly, that the evolving population is in linkage equilibrium in every generation, i.e., that $D_n = 0$ for all n, and then this inexact model will be compared with the exact treatment in which no such assumption is made.

With two loci and two alleles at each locus all possible genotypes can be tabulated in a two-way table of nine cells, in each of which are found the frequency and adaptive value of the individual genotypes (table 1). The frequency of each genotype is denoted by Z_{ij}, and the adaptive value by W_{ij}. The latter is the relative probability that a zygote of this genotype will leave a zygote in the next generation. The subscript, i, stands for the number of alleles, A, and j for the number of alleles, B, in each genotype. The mean adaptive value of the population is obtained from Table 1 as

$$\bar{W} = \sum_{i,\,j=0}^{2} Z_{ij} \cdot W_{ij}. \tag{4}$$

TABLE 1. *The frequencies (the upper entries) and adaptive values (the lower entries) of all possible genotypic arrays with two loci (see text)*

	AA	Aa	aa	Marginal mean
BB	Z_{22}	Z_{12}	Z_{02}	$\bar{W}_{BB}$
	W_{22}	W_{12}	W_{02}	
Bb	Z_{21}	Z_{11}	Z_{01}	$\bar{W}_{Bb}$
	W_{21}	W_{11}	W_{01}	
bb	Z_{20}	Z_{10}	Z_{00}	$\bar{W}_{bb}$
	W_{20}	W_{10}	W_{00}	
Marginal mean	$\bar{W}_{AA}$	$\bar{W}_{Aa}$	$\bar{W}_{aa}$	$\bar{W}$

Under the assumptions of random mating and of linkage equilibrium at every generation, the Z_{ij}'s are the products of the appropriate gametic frequencies in formulae (1). For example, $Z_{22} = p^2r^2$, $Z_{21} = 2p^2r(1 - r)$ and so on. From these and the set of W_{ij} given, it is possible to calculate the mean adaptive value, $\bar{W}$, for any combination of gene frequencies, p and r. These mean adaptive values may then be put in the form of *adaptive topography*, a concept introduced by Wright (1932). Figure 1 shows a topography taken from Lewontin and White (1960). The two axes represent the two gene frequencies p and r. The lines in the plane (*isodapts*) join points of equal $\bar{W}$ in the manner of lines of equal altitude in a topographic map. Two adaptive peaks marked by P, two valleys marked by V and a saddle point, S, are seen in figure 1.

It has been shown by Wright (1942) and others that in the simple case of linkage equilibrium the peaks represent points of stable gene frequency equilibrium, while the saddle or minimax point is one of unstable equilibrium. That is, in a population the gene frequencies will change under the influence of natural selection until they arrive at a peak, and, although the frequencies may remain in equipoise at a saddle point, any slight disturbance will cause them to change toward a peak. In the case of two peaks, as in figure 1, the particular peak to which the frequencies move, depends upon the initial frequencies. The genetic conditions for a stable gene frequency equilibrium when linkage disequilibrium is unimportant are given by Kojima (1959).

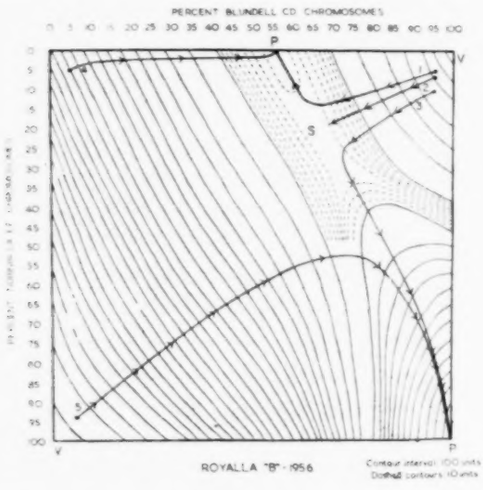

FIG. 1. Adaptive topography for Royalla population of *Moraba scurra* taken from Lewontin and White (1960). The abscissa is the frequency of the Blundell inversion on chromosome *CD*, the ordinate is the frequency of the Tidbinbilla inversion on Chromosome *EF*. Lines are those of equal adaptive value (isodapts). The numbered trajectories are the paths of gene frequency change computed from equations 24 *a-c*. *P*, *V*, and *S* show the locations of adaptive peaks, valleys and a saddle respectively.

What is to be examined further here is the trajectory of the population as it approaches equilibrium. This will then be compared with the trajectory and equilibrium situation when linkage equilibrium is no longer assumed.

It has been shown by Wright (1942) that for linkage equilibrium the changes in gene frequency per generation, Δp and Δr, may be written as

$$\Delta p = \frac{1}{2\bar{W}} p(1 - p) \frac{\delta \bar{W}}{\delta p} \qquad (5.a)$$

$$\Delta r = \frac{1}{2\bar{W}} r(1 - r) \frac{\delta \bar{W}}{\delta r} \qquad (5.b)$$

or in the case of a continuous change of gene frequency with time,

$$\frac{dp}{dt} = \tfrac{1}{2}p(1 - p) \frac{\delta \bar{W}}{\delta p} \qquad (6.a)$$

$$\frac{dr}{dt} = \tfrac{1}{2}r(1 - r) \frac{\delta \bar{W}}{\delta r}. \qquad (6.b)$$

Combining (6.a) and (6.b) to eliminate dt, yields,

$$r(1 - r) \frac{\delta \bar{W}}{\delta r} dp = p(1 - p) \frac{\delta \bar{W}}{\delta p} dr. \qquad (7)$$

By integrating both sides of (7), an equation for the trajectory of p and r in the gene frequency plane can be obtained. This equation will contain the initial gene frequencies, p_0 and r_0, which

determine the specific paths of gene frequency changes. To see the significance of the equation (11), it is fruitful to analogize the path of genetic evolution with the path of a particle moving in a two-dimensional potential field. The coordinates of the particle are the p- and r-axes, and the isodapts are equipotential lines. This type of analogy has been explored to some extent by Wright (1955) and Lewontin (1958) for a single locus case. In such a potential field a particle will move so as to make the maximum change of potential per unit length of path. This can be represented in the symbolism used in (7), by the differential equation

$$\frac{\delta \bar{W}}{\delta r}\, dp = \frac{\delta \bar{W}}{\delta p}\, dr, \qquad (8)$$

and the rate of change of potential along this path is

$$\sqrt{\left[\frac{\delta \bar{W}}{\delta r}\right]^2 + \left[\frac{\delta \bar{W}}{\delta p}\right]^2}. \qquad (9)$$

(Proofs of (8) and (9) may be found in any elementary text of calculus in discussions of directional derivatives and gradients.) Now a comparison of (7) and (8) shows that gene frequencies do *not* change in such a way that the rate of increase of $\bar{W}$ is maximized, although eventually the population does reach a point of maximum mean adaptive value (Wright, 1949). In a sense the population takes an "indirect" path to the peak. It is to be emphasized that the actual position of a population in the gene frequency plane as well as the gradient in fitness determines the vectors of gene frequency displacement and the change of mean adaptive value is only a resultant of gene frequency changes.

To illustrate the difference between the "steepest path" predicted by (8) and the "genetic path" given by (7) we have solved these equations for two cases. In Case I, the adaptive values which are given in table 2, show no epistasis, while for Case II given in table 3 there is a strong epistasis or non-allelic interaction between the two loci. In figure 2 the steepest path (solid curve) and the genetic path (dashed curve) are given for Case I. Several sets of paths are shown, each corresponding to different initial values of p and r. Because of the symmetry of the adaptive topography with one central peak at $p = r = .50$, only one quadrant of the

TABLE 2. *Hypothetical fitness values of nine genotypes of locus A and locus B. No epistasis*

	AA	*Aa*	*aa*
BB	1	3	1
Bb	2	4	2
bb	1	3	1

TABLE 3. *Hypothetical fitness values showing epistasis between locus A and locus B*

	AA	*Aa*	*aa*
BB	1	1.5	1
Bb	2.5	4	2.5
bb	1	1.5	1

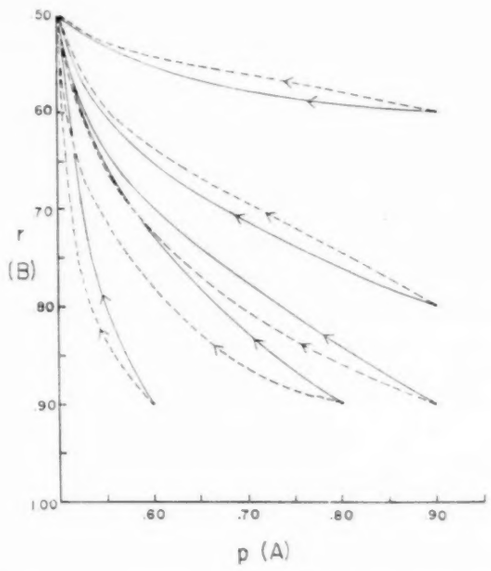

FIG. 2. Steepest path (solid lines) and path taken by gene frequencies with constant linkage equilibrium (broken lines) for adaptive values in table 2 (no epistasis). Ordinate and abscissa are frequencies of alleles at two loci.

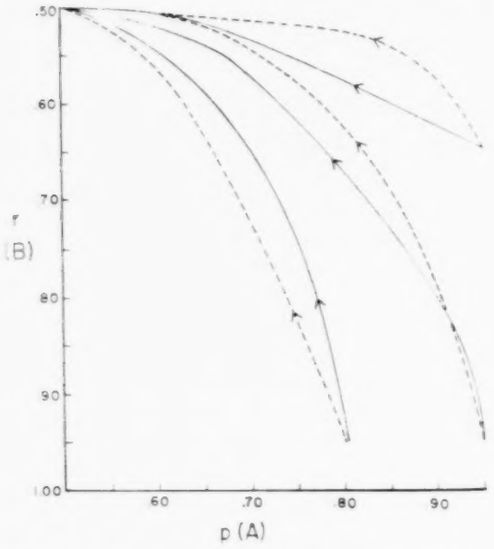

FIG. 3. Steepest path (solid lines) and path taken by gene frequencies with constant linkage equilibrium (broken lines) for the adaptive values in table 3 (epistasis). Ordinate and abscissa are frequencies of alleles at two loci.

p—r plane is shown. The results of calculations for Case II are shown in figure 3. The deviations of the genetic paths from the steepest paths are in the direction of a larger vector component for that gene frequency closer to .50. The paths are more divergent for the epistatic case than for the non-epistatic, these deviations being in some cases quite large.

One of the most interesting concomitants of this effect arises when there is more than one adaptive peak in the topography as in figure 1. In such cases the population may actually go to a different peak than that which has the *steepest gradient* on the adaptive topography. This complication is in addition to the fact that the population does not necessarily ascend the *highest* peak, since gene frequencies only move to points of *local* maxima of mean adaptive values. This latter point can be seen in the trajectory of figure 1. Depending upon initial conditions, the populations may go to either peak or toward the saddle.

THE COMPLICATION OF LINKAGE

When the assumption of constant linkage equilibrium is relaxed, which is necessary for an accurate treatment of the problem, it is gametic rather than gene frequencies that must be examined. Using the notations developed in the previous sections, let $g_{kl}^{(t)}$, $k = 1$ or 0 and $l = 1$ or 0, be the frequency of a particular gametic type in the t-th generation immediately after meiosis. Then $Z_{ij}^{(t)}$ will be the frequency of a zygote formed from the gametes $g_{kl}^{(t)}$'s, while $g_{kl}^{(t+1)}$ denotes the frequency of a particular gamete formed by meiosis of the zygotes in the t-th generation. In table 4 the relations between the $Z_{ij}^{(t)}$ and the $g_{kl}^{(t)}$ are given in the second and third columns for a random mating population. The relative frequencies of these ten genotypes after selection are given in the fifth column. After selection, meiosis occurs again to produce the gametes of the $(t+1)$-th generation The gametic frequencies, $g_{kl}^{(t+1)}$, are easily computed with the aid of the last column of table 4. The gametic frequencies in the $(t+1)$-th generation are then:

$$g_{11}^{(t+1)} = \frac{g_{11}^t g_{11}^t W_{22} + \frac{1}{2}(2g_{11}^t g_{10}^t W_{21} + 2g_{11}^t g_{01}^t W_{12}) + [(1-R)g_{11}^t g_{00}^t + Rg_{10}^t g_{01}^t]W_{11}}{\bar{W}} \quad (10.a)$$

$$g_{10}^{(t+1)} = \frac{g_{10}^t g_{10}^t W_{20} + \frac{1}{2}(2g_{11}^t g_{10}^t W_{21} + 2g_{10}^t g_{00}^t W_{10}) + [Rg_{11}^t g_{00}^t + (1-R)g_{10}^t g_{01}^t]W_{1.}}{\bar{W}} \quad (10.b)$$

$$g_{01}^{(t+1)} = \frac{g_{01}^t g_{01}^t W_{02} + \frac{1}{2}(2g_{01}^t g_{11}^t W_{12} + 2g_{01}^t g_{00}^t W_{01}) + [Rg_{11}^t g_{00}^t + (1-R)g_{10}^t g_{01}^t]W_{11}}{\bar{W}} \quad (10.c)$$

$$g_{00}^{(t+1)} = \frac{g_{00}^t g_{00}^t W_{00} + \frac{1}{2}(2g_{00}^t g_{10}^t W_{10} + 2g_{00}^t g_{01}^t W_{01}) + [(1-R)g_{11}^t g_{00}^t + Rg_{10}^t g_{01}^t]W_{11}}{\bar{W}}. \quad (10.d)$$

TABLE 4. *Relations between gametic and genotypic frequencies in a random mating population*

Genotype	Zygotic frequency	Composition	Adaptive value	Frequency after selection	Gametes produced
$\frac{AB}{AB}$	$Z_{22}^{(t)}$	$g_{11}^{(t)}g_{11}^{(t)}$	W_{22}	$g_{11}^{(t)}g_{11}^{(t)}W_{22}/\bar{W}$	$1AB$
$\frac{AB}{Ab}$	$Z_{21}^{(t)}$	$2g_{11}^{(t)}g_{10}^{(t)}$	W_{21}	$2g_{11}^{(t)}g_{10}^{(t)}W_{21}/\bar{W}$	$\frac{1}{2}AB$ $\frac{1}{2}Ab$
$\frac{Ab}{Ab}$	$Z_{20}^{(t)}$	$g_{10}^{(t)}g_{10}^{(t)}$	W_{20}	$g_{10}^{(t)}g_{10}^{(t)}W_{20}/\bar{W}$	$1Ab$
$\frac{AB}{aB}$	$Z_{12}^{(t)}$	$2g_{11}^{(t)}g_{01}^{(t)}$	W_{12}	$2g_{11}^{(t)}g_{01}^{(t)}W_{12}/\bar{W}$	$\frac{1}{2}AB$ $\frac{1}{2}aB$
$\frac{AB}{ab}$	$Z_{11}^{(t)}$	$2g_{11}^{(t)}g_{00}^{(t)}$	W_{11}	$2g_{11}^{(t)}g_{10}^{(t)}W_{11}/\bar{W}$	$\frac{1}{2}(1-R)AB$, $\frac{1}{2}R\,Ab$ $\frac{1}{2}R\,aB$, $\frac{1}{2}(1-R)ab$
$\frac{Ab}{bB}$		$2g_{10}^{(t)}g_{01}^{(t)}$	W_{11}	$2g_{10}^{(t)}g_{01}^{(t)}W_{11}/\bar{W}$	$\frac{1}{2}R\,AB$, $\frac{1}{2}(1-R)Ab$ $\frac{1}{2}(1-R)aB$, $\frac{1}{2}R\,ab$
$\frac{Ab}{ab}$	$Z_{10}^{(t)}$	$2g_{10}^{(t)}g_{00}^{(t)}$	W_{10}	$2g_{10}^{(t)}g_{00}^{(t)}W_{10}/\bar{W}$	$\frac{1}{2}Ab$ $\frac{1}{2}ab$
$\frac{aB}{aB}$	$Z_{02}^{(t)}$	$g_{01}^{(t)}g_{01}^{(t)}$	W_{02}	$g_{01}^{(t)}g_{01}^{(t)}W_{02}/\bar{W}$	$1aB$
$\frac{aB}{ab}$	$Z_{01}^{(t)}$	$2g_{01}^{(t)}g_{00}^{(t)}$	W_{01}	$2g_{01}^{(t)}g_{00}^{(t)}W_{01}/\bar{W}$	$\frac{1}{2}aB$ $\frac{1}{2}ab$
$\frac{ab}{ab}$	$Z_{00}^{(t)}$	$g_{00}^{(t)}g_{00}^{(t)}$	W_{00}	$g_{00}^{(t)}g_{00}^{(t)}W_{00}/\bar{W}$	$1ab$

Making the convention that W_{AB} is the average adaptive value of gametic phase AB in all its combinations, i.e., *the the marginal mean* of the gamete AB, and similarly W_{aB}, W_{Ab} and W_{ab} for AB, Ab and ab, respectively, the four equations from (10.a) to (10.d) can be written in the form

$$g_{11}^{(t+1)} = \frac{g_{11}^{t}W_{AB} - RW_{11}D^{t}}{\bar{W}} \quad (11.a)$$

$$g_{10}^{(t+1)} = \frac{g_{10}^{t}W_{Ab} + RW_{11}D^{t}}{\bar{W}} \quad (11.b)$$

$$g_{01}^{(t+1)} = \frac{g_{01}^{t}W_{aB} + RW_{11}D^{t}}{\bar{W}} \quad (11.c)$$

$$g_{00}^{(t+1)} = \frac{g_{00}^{t}W_{ab} - RW_{11}D^{t}}{\bar{W}}. \quad (11.d)$$

Finally, the changes in gametic frequencies in each generation, Δg_{kl}, are expressed as follows (dropping the superscripts),

$$\Delta g_{11} = \frac{g_{11}(W_{AB} - \bar{W}) - RW_{11}D}{\bar{W}} \quad (12.a)$$

$$\Delta g_{10} = \frac{g_{10}(W_{Ab} - \bar{W}) + RW_{11}D}{\bar{W}} \quad (12.b)$$

$$\Delta g_{01} = \frac{g_{01}(W_{aB} - \bar{W}) + RW_{11}D}{\bar{W}} \quad (12.c)$$

$$\Delta g_{00} = \frac{g_{00}(W_{ab} - \bar{W}) - RW_{11}D}{\bar{W}}. \quad (12.d)$$

Equations (12.a)–(12.d) are equivalent to those derived by Kimura (1956) in his treatment of a special case of inter-

action between two genes. His derivation was based on a time-continuous model (instead of generation-time) with instantaneous rates of birth and death.

The Equilibrium Condition

First, to be examined is the question of whether the complication of linkage disturbs the simple equilibrium picture expected with the assumption of constant linkage equilibrium. Specifically, do the gene frequencies come to the same equilibrium values, irrespective of linkage, and does the population reach linkage equilibrium as it approaches equilibrium?

An equilibrium of the population in the present investigation is defined by

$$\Delta g_{11} = \Delta g_{10} = \Delta g_{01} = \Delta g_{00} = 0. \quad (13)$$

Under this definition not only do the frequencies of gametes and zygotes not change but also the value of D must be a constant. Substituting (13) in equations (12.a)–(12.d), yields

$$g_{11}(W_{AB} - \bar{W}) - RDW_{11} = 0 \quad (14.a)$$

$$g_{10}(W_{ab} - \bar{W}) + RDW_{11} = 0 \quad (14.b)$$

$$g_{01}(W_{ab} - \bar{W}) + RDW_{11} = 0 \quad (14.c)$$

$$g_{00}(W_{ab} - \bar{W}) - RDW_{11} = 0. \quad (14.d)$$

These deceptively simple equations are not easy to solve in general since they are cubic equations in the g_{kl}'s. By examining some symmetrical cases of adaptive values, however, the equations can be rendered simple enough to solve. Specifically, we have used adaptive values given in table 5 where a, b, c, and d can, in general, take any value. This model allows epistasis when

$$a - b - c + d \neq 0.$$

Table 5. *General scheme of adaptive values for the symmetrical case*

	AA	*Aa*	*aa*
BB	*a*	*b*	*a*
Bb	*c*	*d*	*c*
bb	*a*	*b*	*a*

In this model the generality is restricted, but not to a great extent when one quadrat in the gene frequency plane, say p and r both being from 1.00 to .50, is considered. Substituting the adaptive values in table 5 into the equations, (14.a)–(14.d), and writing them *in extenso* gives:

$$g_{11}[ag_{11}+cg_{10}+bg_{01}+dg_{00}-\bar{W}] - Rd[g_{11}g_{00}-g_{10}g_{01}]=0 \quad (15.a)$$

$$g_{10}[cg_{11}+ag_{10}+dg_{01}+bg_{00}-\bar{W}] + Rd[g_{11}g_{00}-g_{10}g_{01}]=0 \quad (15.b)$$

$$g_{01}[bg_{11}+dg_{10}+ag_{01}+cg_{00}-\bar{W}] + Rd[g_{11}g_{00}-g_{10}g_{01}]=0 \quad (15.c)$$

$$g_{00}[dg_{11}+bg_{10}+cg_{01}+ag_{00}-\bar{W}] - Rd[g_{11}g_{00}-g_{10}g_{01}]=0. \quad (15.d)$$

Because of the symmetry of the equations, (15.a) with (15.d) and (15.b) with (15.c), the solutions require that $g_{11} = g_{00}$ and $g_{10} = g_{01} = \frac{1}{2} - g_{11}$. Making the appropriate substitutions in the equations, (15.a), and noting that

$$\bar{W} = 4(a+d-b-c)g_{11}^2 - 2(a+d-b-c)g_{11} + \frac{a+d}{2} \quad (16)$$

it is found that

$$g_{11}(b+c-d-a)(4g_{11}^2-3g_{11}+\tfrac{1}{2}) - Rd(g_{11}-\tfrac{1}{4})=0. \quad (17)$$

In the following, two cases, epistatic and non-epistatic, are to be examined.

Case I: *Additivity between loci*

When $a + d - c - b = 0$, there is no epistasis (see, e.g. Kojima, 1959). If the adaptive values satisfy this condition, then the solution is

$$g_{11} = g_{10} = g_{01} = g_{00} = \tfrac{1}{4}.$$

That is, there is an equilibrium at $p = r = \frac{1}{2}$ and there is no linkage disequilibrium since

$$D = (g_{11}g_{00} - g_{10}g_{01}) = 0$$

The stability of the equilibrium now depends simply on whether the value of

d is the largest among the four adaptive values or not. If it is, then the point, $p = r = \frac{1}{2}$, is a simple overdominant equilibrium.

Case II: *Interaction between loci—epistasis*

If $a + d - b - c \neq 0$, then the equation, (17), is a cubic equation with three distinct solutions. They are:

$$g_{11} = \tfrac{1}{4} + \tfrac{1}{4}\sqrt{1 + \frac{4Rd}{b + c - a - d}} \quad (18.a)$$

$$g_{11} = \tfrac{1}{4} - \tfrac{1}{4}\sqrt{1 + \frac{4Rd}{b + c - a - d}} \quad (18.b)$$

$$g_{11} = \tfrac{1}{4}. \quad (18.c)$$

Since $g_{11} = g_{00} = \frac{1}{2} - g_{01} = \frac{1}{2} - g_{10}$, the gene frequencies of both loci, p and r, are again $\frac{1}{2}$ at equilibrium. But if (18.a) or (18.b) should be the solution, there would *no longer be linkage equilibrium*, because

$$D = g_{11}g_{00} - g_{10}g_{01} = \pm \tfrac{1}{4}\sqrt{1 + \frac{4Rd}{b + c - d - a}}. \quad (19)$$

In order for (18.a) and (18.b) to hold true, the quantity under the radical sign must be between zero and one, inclusive. This requires that $b + c - a - d$ be negative and that

$$R \leq \frac{a + d - b - c}{4d}. \quad (20)$$

That is, linkage must be tighter than the value on the right-hand side of (20), or else the system will go to linkage equilibrium given in (18.c). For example, using values in Table 3, $a = 1$, $b = 1.5$, $c = 2.5$, $d = 4$, R must then be less than .0625 for permanent linkage disequilibrium. When $R = .0625$, three solutions, (18.a)–(18.c) are equal.

Finally, there is the possibility that (18.a) and/or (18.b) represent unstable equilibrium and only (18.c) is stable, or the reverse. The stability of equilibria in this case can be tested by regarding the four gametic types as four alleles of a single super-locus. The rules for testing stability of this type of equilibria are given by Kimura (1956). The algebra is extensive but straightforward and will not be reproduced here in any detail.

We simply point out that for (18.c) to be a stable equilibrium it is necessary for the matrix

$$\begin{vmatrix} 2(a-d) & (a+c-b-d) & (a+b-c-d) \\ (a+c-b-d) & 2(a-b)-4Rd & (a+d-b-c)-4Rd \\ (a+b-c-d) & (a+d-b-c) & 2(a-c)-4Rd \end{vmatrix}$$

to be negative definite. This turns out to be equivalent to requiring that

$$R > \frac{a + d - b - c}{4d} \quad (21.a)$$

and

$$|a - d| > |b - c| \quad \text{and} \quad d > a. \quad (21.b)$$

That is to say, these conditions are necessary and sufficient for an equilibrium at $g_{11} = g_{10} = g_{01} = g_{00} = \frac{1}{4}$ to be stable. Since the condition (21.a) is the converse of the inequality, (20), linkage equilibrium at $p = r = \frac{1}{2}$ for the model given in Table 5 will represent a stable equilibrium of the population if and only if (21.a) and (21.b) are true. The condition, (21.a) assures that the population tends to linkage equilibrium as it approaches gene frequency equilibrium. The stability of such equilibria was studied by Kojima (1959): the necessary and sufficient conditions for such equilibria to be stable are (1) overdominance on the marginal means of three zygotic phases at each locus and (2) additive × additive epistatic variance being smaller than dominance variance. In the present model, the condition, (21.b), turns out to be the same as overdomi-

nance, i.e., $\bar{W}_{Aa} > \bar{W}_{AA}$, $\bar{W}_{Aa} > \bar{W}_{aa}$, $\bar{W}_{Bb} > \bar{W}_{BB}$ and $\bar{W}_{Bb} > \bar{W}_{bb}$ (see Table 1). The second condition on the magnitude of additive × additive epistasis is also satisfied in this case, since the additive × additive variance is zero at $g_{11} = g_{10} = g_{01} = g_{00} = \frac{1}{4}$.

When the inequality, (20), is true, linkage may be so tight that linkage equilibrium would not be achieved in the population. If there is an equilibrium under this condition, the solutions of gametic frequencies in (18.a) and/or (18.b) represent those at equilibrium. The amount of linkage disequilibrium in such a case is given in (19). It is extremely difficult to obtain the *critical upper bound of* R with which populations tend to a stable equilibrium without linkage equilibrium, although, when the values of fitness and recombination fractions are given, the stability of such equilibria is readily tested.

Consider the adaptive values of table 3. It has already been found that with recombination fractions greater than .0625, the population tends to a stable equilibrium at $g_{11} = g_{10} = g_{01} = g_{00} = \frac{1}{4}$. If $R = .05$, then

$$\hat{g}_{11} = \hat{g}_{00} = \tfrac{1}{4} \pm \tfrac{1}{4}\sqrt{1-16R} = .362 \text{ or } .138$$

$$\hat{g}_{01} = \hat{g}_{10} = \tfrac{1}{4} \mp \tfrac{1}{4}\sqrt{1-16R} = .138 \text{ or } .362$$

$$D = \pm\tfrac{1}{4}\sqrt{1-16R} = \pm .112.$$

Both of these equilibria turn out to be stable. When R tends to zero, the disequilibrium tends increasingly to $\pm\frac{1}{4}$ which is the maximum value for D. Using Table 1 it can be shown that at equilibrium

$$\bar{W}_{Aa} > \bar{W}_{AA} \quad \text{and} \quad \bar{W}_{aa}$$

and

$$\bar{W}_{Bb} > \bar{W}_{BB} \quad \text{and} \quad \bar{W}_{bb}.$$

The next example is a model with $a = 3$, $b = 4$, $c = 1$, and $d = 5$ which shows epistasis. In this case there is *no* stable equilibrium with linkage equilibrium since these adaptive values violate the condition (21.b). The converse of the condition, (21.a), i.e.,

$$R \leq \frac{a + d - b - c}{4d}$$

becomes

$$R \leq .15.$$

However, the numerical computation shows that equilibria are not stable unless R is less than approximately .116. In other words, the model under present consideration does not lead to any stable equilibrium unless linkage is tighter than .116. Computing $\bar{W}_{AA}$, $\bar{W}_{Aa}$, etc. appearing in table 1, it is found that these marginal means satisfy overdominance at such stable equilibria. Findings from this and several other examples suggest that over-dominance at equilibrium is necessary for populations to be maintained in a stable equilibrium, just as in the case of the epistatic model in linkage equilibrium (Kojima, 1959).

The effect of the linkage disequilibrium on the mean adaptive value can be judged from the expression, (16), which can be rewritten as

$$\bar{W} = 4D^2(a+d-b-c) + \frac{a+b+c+d}{4}. \quad (22)$$

At equilibrium the difference in $\bar{W}$ with and without linkage disequilibrium is

$$4D^2(a + d - b - c)$$

which is not large unless the epistatic deviation measured by

$$(a + d - b - c)$$

is extremely large. Moreover, the size of $\bar{W}$ does not depend on the sign of D. That is, the excess of coupling or repulsion phase in the case of symmetrical adaptive values given in table 5 is immaterial.

It might be supposed that results so far presented are restricted to the relatively simple symmetrical set of adaptive values shown in table 5. This set was chosen as an illustration because analytic

TABLE 6. *Hypothetical fitness values for a partially asymmetrical case showing the same degree of epistasis as the symmetrical case in Table 3*

	AA	*Aa*	*aa*
BB	1	1.5	1
Bb	2.5	4	2.5
bb	2	2.5	2

solutions to the equilibrium equations are possible for a symmetrical case. For an asymmetrical case although analytic solutions are not possible, numerical solutions can be found, and we have included a case of this sort to show the generality of our result. Table 6 gives the adaptive values for a case in which there is symmetry with respect to the A locus but asymmetry with respect to the B locus. The epistatic deviations are identical with these in table 3. The results of numerical calculation for this new case are given in table 7. As the table shows, not only are there profound effects of linkage on the gametic frequencies but in this case there are also marked effects or *gene frequencies*. This latter phenomenon did not appear in the symmetrical case. Again, however, rather tight linkage must be assumed before there is any departure from the expected result with no linkage complication. The critical upper bound for R cannot be given analytically but it appears to be close to if not equal to .0625 as in the previous case. For R as large as .08 the stable equilibrium condition is that predicted if linkage is ignored. The smaller the value of recombination the greater is the departure from this expectation both in gametic and gene frequencies and as in the previous examples two stable, conjugate, equilibria exist for each condition. This occurrence of paired solutions is a result of the symmetry of the A locus since in a sense, A and a are indistinguishable.

THE APPROACH TO EQUILIBRIUM

Although a fairly marked epistasis associated with a tight linkage is required to disturb linkage equilibrium and gene frequency equilibrium, the approach to the equilibrium will be affected by any values of linkage and of fitness. To investigate these effects, the equations, (12.a)–(12.d), are put in their approximate differential forms:

$$\frac{dg_{11}}{dt} = g_{11}(W_{AB} - \bar{W}) - RDW_{11} \quad (23.a)$$

$$\frac{dg_{10}}{dt} = g_{10}(W_{Ab} - \bar{W}) + RDW_{11} \quad (23.b)$$

$$\frac{dg_{01}}{dt} = g_{01}(W_{aB} - \bar{W}) + RDW_{11} \quad (23.c)$$

TABLE 7. *Equilibrium values of gametic frequencies, gene frequencies, adaptive value and linkage disequilibrium for the fitnesses shown in table 6. R is the value of recombination*

R	g_{11}	g_{10}	g_{01}	g_{00}	p	r	$\bar{W}$	D
.01	.014	.576	.376	.034	.590	.390	2.762	−.216
	.376	.034	.014	.576	.410	.390	2.762	+.216
.02	.030	.549	.351	.071	.579	.381	2.724	−.190
	.351	.071	.030	.549	.421	.381	2.724	+.190
.04	.068	.482	.289	.161	.550	.357	2.652	−.128
	.289	.161	.068	.482	.450	.357	2.652	+.128
.062	.156	.348	.179	.317	.504	.335	2.584	−.013
	.179	.317	.156	.348	.496	.335	2.584	+.013
.08	.167	.333	.167	.333	.500	.333	2.582	.000

$$\frac{dg_{00}}{dt} = g_{00}(W_{ab} - \bar{W}) - RDW_{11}. \quad (23.d)$$

The factor of time can then be eliminated by dividing (23.a), (23.b) and (23.c) by (23.d), yielding

$$\frac{dg_{11}}{dg_{00}} = \frac{g_{11}(W_{AB} - \bar{W}) + RDW_{11}}{g_{00}(W_{ab} - \bar{W}) + RDW_{11}} \quad (24.a)$$

$$\frac{dg_{10}}{dg_{01}} = \frac{g_{10}(W_{Ab} - \bar{W}) - RDW_{11}}{g_{00}(W_{ab} - \bar{W}) + RDW_{11}} \quad (24.b)$$

$$\frac{dg_{01}}{dg_{00}} = \frac{g_{01}(W_{aB} - \bar{W}) - RDW_{11}}{g_{00}(W_{ab} - \bar{W}) + RDW_{11}}. \quad (24.c)$$

When these equations are written in their extensive forms in terms of adaptive values of genotypes and solved simultaneously, the trajectories of gametic frequency changes can be plotted, and these, in turn, can be put in terms of gene frequencies by adding the appropriate gametic frequencies. The simultaneous equations, (24.a), (24.b) and (24.c), are not easy to solve analytically, so numerical solutions for several cases have been obtained by using a combination of the Runge-Kutta and Milne methods adapted to digital computers. Specifically, we have used the two sets of adaptive values given in tables 2 and 3 in combination with different recombination fractions. The results are shown in figures 4 and 5. The trajectories under the assumption of linkage equilibrium are included for comparison. In each case the initial gametic frequencies were such that the populations started in linkage equilibrium.

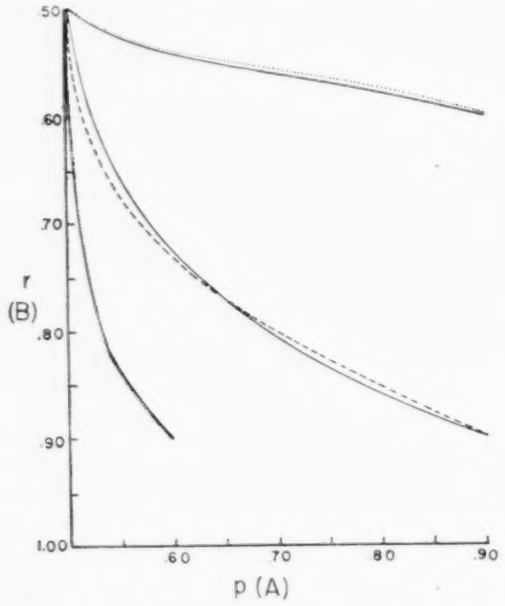

FIG. 4. Trajectories of gene frequencies under the assumption of constant linkage equilibrium (solid lines) compared with true trajectories for $R = .1250$ (dashed lines) and $R = .0000$ (dotted lines). Non-epistatic case of table 2.

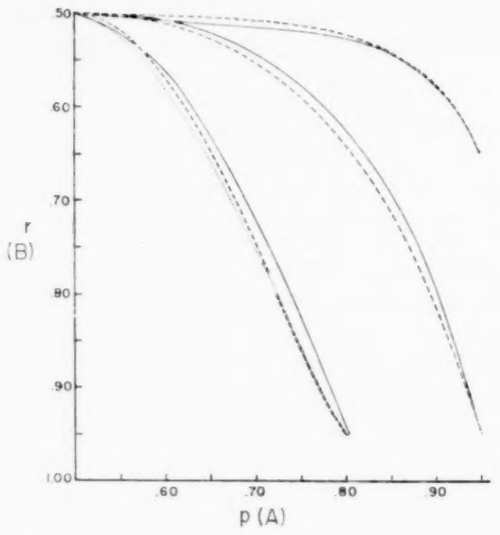

FIG. 5. Trajectories of gene frequency under the assumption of constant linkage equilibrium (solid lines) compared with true trajectories for $R = .1250$ (dashed lines) and $R = .0000$ (dotted lines). Epistatic case of table 3.

Any deviation from the simpler case is then due to an accumulation of linkages during the process of selection. As the curves show, there is a real, but small, difference between the curves computed with and without the linkage equilibrium assumption. Again it is borne out that there may not be a great effect on gene frequencies due to linkage.

There is, however, a profound effect on gametic frequencies. Figure 6 shows this effect clearly. It is a plot of the values of D against the changing frequency of the allele, A, for the epistatic case with initial conditions $p = .80$ and $r = .95$. The largest value that D can

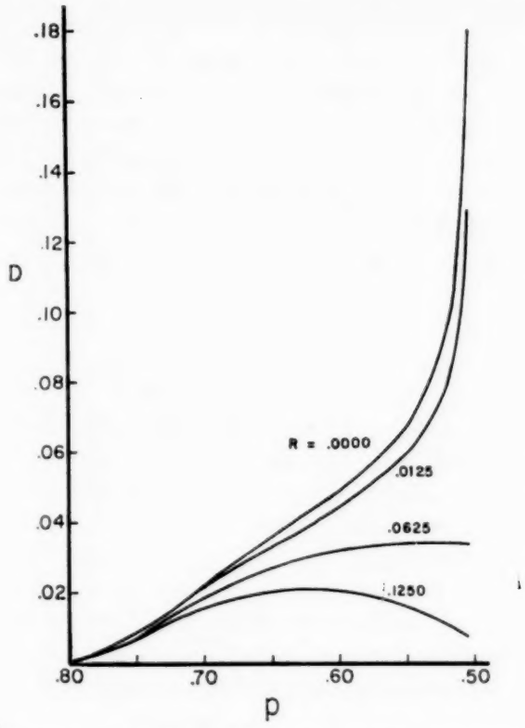

FIG. 6. Changes in the value of D, the linkage disequilibrium, during the process of gene frequency change. The trajectory is the lowermost one in figure 5 (epistatic case). Ordinate shows values of D corresponding to gene frequency, p, of allele A on the abscissa.

ever attain is $\pm.25$ which would correspond to only coupling or only repulsion gametes. As the figure shows, very tight linkages result in a constantly increasing value of D up to the limit of .25. When $R = .0625$ which separates equilibria with and without linkage disequilibrium, D goes as high as .036. For $R = .125$ a maximum is reached at $D = .02$ and then D declines toward zero at gene frequency equilibrium. No points are given at $p = .50$, because the method of solution becomes unstable at the point where the derivatives do not exist. For this reason the curves for $R = .0625$ and $R = .125$ are not shown decreasing to zero although they do come down sharply toward zero near $p = .50$.

A qualitatively similar picture is obtained for the additive case except that even with extremely tight linkage the value of D never exceeds 10^{-7}. This suggests that linkage among non-interacting genes does not build up disequilibrium of linkage.

Whether D will be positive or nega-

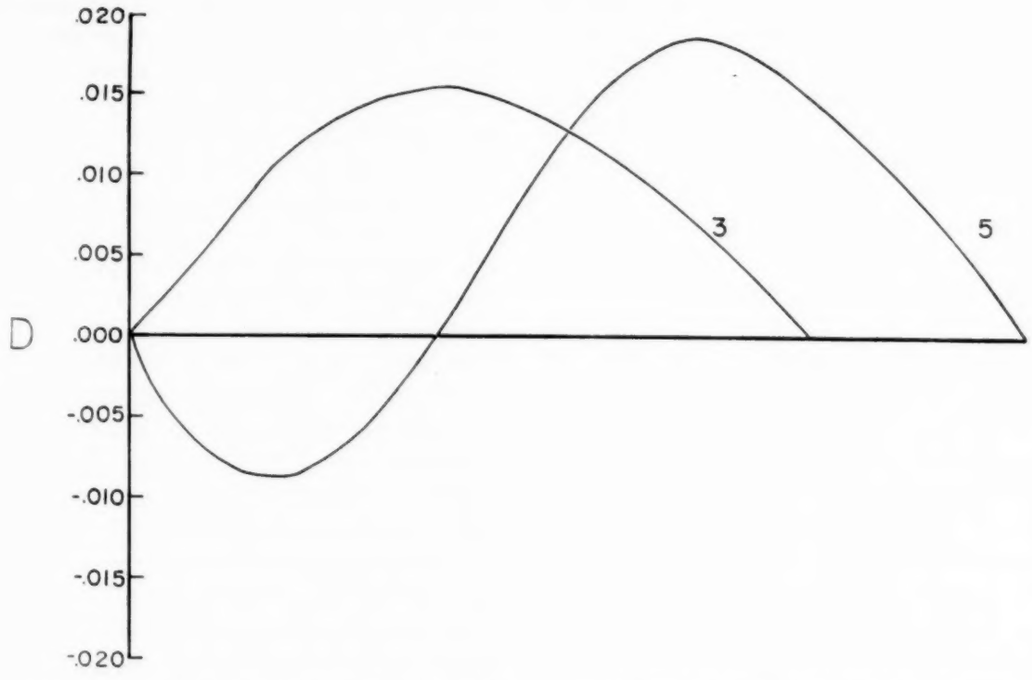

FIG. 7. Values of the linkage disequilibrium, D, on the ordinate, plotted against distance along the trajectory in arbitrary units for trajectories 3 and 5 of figure 1. $R = .50$.

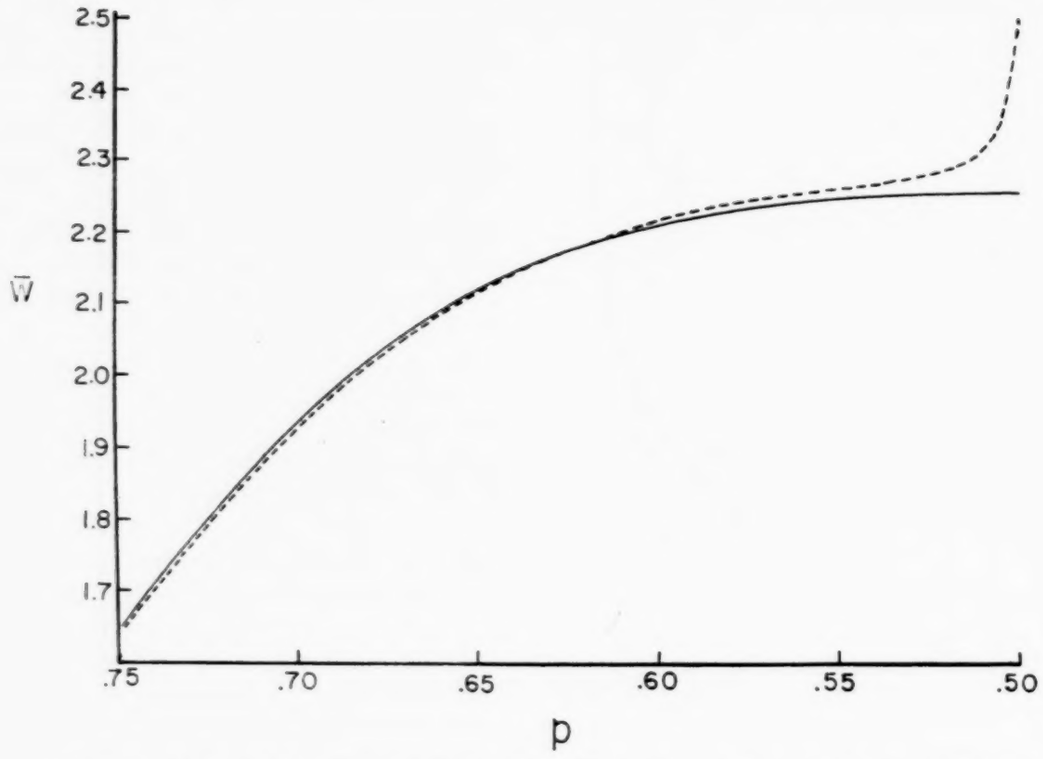

FIG. 8. Values of the mean adaptive value, $\bar{W}$, (ordinate) plotted against successive gene frequencies of allele A for the lowermost trajectory of figure 5 (epistatic case). Solid line is $\bar{W}$ for linkage equilibrium, broken line is $\bar{W}$ for $R = .0000$.

tive, and whether increasing or decreasing in any generation will depend upon the instantaneous value of D, the recombination fraction, the adaptive values of genotypes and the particular path on the adaptive surface which is being traversed by the gene frequencies. As an example of the complexity of changes possible in D, figure 7, has been drawn. Figure 1 shows a variety of evolutionary trajectories computed for the complex adaptive surface. Corresponding to trajectory 3 and 5 in figure 1, a plot of changes in D has been made in figure 7. On the ordinate of figure 7 are the values of D and on the abscissa are values of the distance along the trajectory from the starting point. The most interesting of these graphs is that for trajectory 5 in which D becomes progressively negative, reaches a minimum value, then rises, becoming positive, to a maximum value and finally declines to zero.

Lastly the values of $\bar{W}$ may be compared between different linkage values. As figure 8 shows, there is virtually no difference in $\bar{W}$ along the trajectory between tight and loose linkages except very near to equilibrium. At this point there is a sudden rise in mean adaptive value in the tightly linked case, but again this amounts to no more than 10%.

GENERAL CONCLUSIONS

It must be said that as a general rule joint effects of linkage and epistasis do not produce serious changes of population structure except under special circumstances. These circumstances are the simultaneous existence of marked epistasis and tight linkage. For example, in order for there to be any important effect of linkage and epistasis among genes on different chromosomes ($R = .50$); the epistatic deviation, $a + d - b - c$, must be greater than twice the fitness of the double heterozygotes, d. This is equivalent to demanding that the fitness, a, of the four double homo-

zygotes be slightly greater than that of the double heterozygote, while the fitness of the four single homozygotes be virtually zero, provided that fitnesses, *a*, *b*, *c* and *d* are all positive. This would result in four steep adaptive peaks at the four corners of the adaptive landscape and one rather shallow peak at the center.

There does, in fact, seem to be a case of this extreme type of epistasis in nature. Levitan and Salzano (1959) have described two linked systems of inversions, *E* and *H*, on the fourth chromosome of *D. guaramunú*. Although there are some ambiguities in classification (*HH* and *hh* homozygotes are indistinguishable), the data show a great excess of double heterozygotes and double homozygotes with a virtual absence of the four single homozygotes. Data on crossing-over between the *E* and *H* systems are not available directly, but linkage seems to be tight. This is then a combination of moderately tight or tight linkage and marked epistasis which produces a most aberrant zygotic array.

When there is tight linkage with the more moderate degree of epistasis, the main effect is on gametic rather than gene frequencies. However, the importance of this effect should not be underestimated because it is the frequencies of the gametes that determine the zygotic frequencies and thus the phenotypic composition of the population. Thus, as in the two epistatic models discussed previously, one can expect a considerable change in population structure when tight linkage is introduced. In the first epistatic model, the frequency of the genotype, *AABB*, would be .0625 at equilibrium if there were no tight linkage, while it is .131 or more than twice as great if recombination fraction is .05. In the second epistatic model, there would *not be any stable equilibrium* and the population would tend to fixation if there were no linkage complication. Gene frequencies alone do not give an adequate picture of the genotypic constitution of the population in such cases and attention must be paid to the gametic frequencies.

An example of permanent linkage disequilibrium in a naturally occurring polymorphism comes from the work of Levitan (1958) on the X chromosome inversions in *Drosophila robusta*. Each arm of the chromosome contains an inverted sequence, *I* and a normal sequence *S*. Crossing over between sequences on the left arm and those on the right arm is infrequent, about one percent. Data on gametic frequencies in males and females are as follows:

	♂	♀
S_LS_R	178	266
S_LI_R	369	587
I_LS_R	176	259
I_LI_R	538	912

These data give values of $D♂ = .019$ and $D♀ = .022$. Such values would not require any extraordinary epistasis to maintain them considering the tightness of linkage coupled with the lack of crossing-over in males. It is difficult to see how such an explanation could be invoked for Levitan's second-chromosome data, however, in which the association observed is not consistently in favor of coupling or repulsion gametes.

To what extent linkage and epistasis are important in nature remains a question, but there is abundant evidence that restriction of crossing-over is a common evolutionary mode. Inversions, translocations, localization of chiasmata and restriction of recombination to one sex are common features in natural populations. When these mechanisms reduce recombination to a low degree, a consideration of the linkage-epistatic interaction becomes important in the study of the evolution of these populations.

Summary

The joint effects of linkage and epistasis (interaction between non-allelic genes in determining fitness) have been

examined for two-locus polymorphisms. The general results are of the following nature:

(1) Gene frequencies change toward a stable equilibrium condition which corresponds to a local maximum in mean adaptive value. This change occurs in such a way that the rate of increase of mean adaptive value is not maximum. That is, the trajectory of gene frequency changes is not the "steepest path" on the adaptive surface.

(2) In the absence of epistasis, linkage does not affect the final equilibrium of the population.

(3) When epistasis is present, linkage must be fairly tight in order for there to be any effect on the final equilibrium. The amount of recombination allowed for such cases is, in general, a function of epistatic deviations.

(4) If linkage is tighter than the value demanded by the magnitude of epistatic deviations, there may be *permanent linkage disequilibrium* of considerable magnitude, and the *gene frequencies* may also be affected.

(5) There are some cases where a stable equilibrium is possible only with a tight linkage. Without such linkage complication, there will be no intermediate gene frequency stable equilibrium.

(6) The approach to the equilibrium condition is affected by linkage irrespective of whether there is epistasis although the effect is greater when epistasis is present.

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PSEUDOGAMY AS AN EVOLUTIONARY FACTOR IN THE POECILIID FISH *MOLLIENISIA FORMOSA*

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Pseudogamy or gynogenesis, the activation of an unfertilized egg by a sperm without the continuing functional participation of male hereditary elements, has long been known in various animals. Thus Peacock (1944) reported that eggs of the worm *Rhabditis monohystera* are activated by sperm of the same species without the participation of male chromosomes. The effect was experimentally secured by Lillie (1912) by mixing homologous eggs and spermatozoa of *Nereis* and separating them after a short time, and similarly by Rothschild (1953) in *Paracentrotus*. The same effect can be achieved across species. Thus much of the early classical work on the activation of frogs' eggs by mechanical means was inspired by the observation by Bataillon in 1909 that eggs of the newt *Pelodytes punctatus* could be pseudogamously activated by spermatozoa of *Triturus alpestris*. Similar observations were made on *Arbacia* and *Chaetopterus* by Godlewski (1912) and on the eggs of the sea urchin *Strongylocentrus* activated by the sperm of a mollusc (*Chlorostoma*) by Loeb (1913). Summaries of the situation have been given by Hertwig (1936) and by Moore (1955).

Few cases seem to have been reported, however, among animals in which parthenogenesis involving the pseudogamous activation of the eggs of one species by the sperm of another constitutes the normal mode of reproduction. There have been correspondingly few opportunities to investigate the impact of this curious reproductive mode in evolution. One of the most interesting situations of this kind in an invertebrate has been reported recently by Moore, Woodroffe, and Sanderson (1956). They have found that the ptinid beetle *Ptinus latro* is an all-female species, the members of which live normally in similar ecological situations with the related bisexual form *P. hirtellus*. Eggs from virgin *latro* females do not hatch, but after mating with *hirtellus* regularly produce typical *latro* females, apparently parthenogenetically. Experiments with males of *hirtellus* sterilized by X rays have shown that viable sperm must participate in the activation, but there is no evidence that genetic elements are involved. Early cytological work suggests that both sexes of *hirtellus* are diploid ($n = 9$), whereas the *latro* females are triploid, and *latro* eggs do not undergo reduction in maturation.

As far as we are aware, the only similar case of naturally occurring pseudogamy in a vertebrate is that first reported by Hubbs (1932, 1933) in the viviparous poeciliid fish *Mollienisia formosa* (Girard), and since abundantly confirmed by Meyer (1938), by Carl Hubbs (1955), by Clark Hubbs, Drewry, and Warburton (1959), and by us. *Mollienisia formosa*, thought by Carl Hubbs to be itself of hybrid origin, is found in northeastern Mexico and southwestern Texas. In the state of Tamaulipas, Mexico, it was found living in fresh waters well inland, in company with the related *M. sphenops* (Cuvier and Valenciennes) species complex. Near Brownsville, Texas, it is an abundant inhabitant of coastal fresh-water pools, living in company with another, related form, *M. latipinna* Le Seur. We have since found populations of *M. formosa* in the states of Tamaulipas and Nuevo León, living sympatrically with populations of the *M. sphenops* complex.

Both *M. sphenops* and its closely related forms, as well as *M. latipinna,* are normal bisexual species. In Tamaulipas, in Nuevo León, and at Brownsville, the wild populations of *M. formosa* examined by Carl Hubbs and Clark Hubbs were found to consist solely of females—a finding entirely confirmed by our own rather extensive examination of similar populations in all three localities. A significant exceptional finding in the Brownsville population has recently been reported by Clark Hubbs, Drewry, and Warburton (1959) which will be discussed more fully later. Carl Hubbs, Meyer, Clark Hubbs, and we have all found that *M. formosa* will not reproduce when separated from the males of *M. latipinna* and *M. sphenops,* which normally accompany it. We have made similar determinations for the populations normally occurring with *M. mexicana* (a member of the *sphenops* complex). When fertilized by any of the three species, abundant broods are produced, evidently pseudogamously, which are normally composed entirely of females phenotypically identical with the mother. Carl Hubbs, Meyer, and Clark Hubbs have all carried this process through many generations with no indication of introgression of male characteristics. We have done the same and, as Carl Hubbs has also done, have accentuated the situation by using conspicuous phenotypic markers, such as dense melanic spotting, in the *sphenops* and *latipinna* males. Our results have been confirmatory, except for one instance which will be described (fig. 1; fig. 2). Thus, as with *Ptinus,* this seems to be a case of a well-defined form living in a kind of reproductive parasitism on a related species through this peculiarly specialized type of natural thelyotokous parthenogenesis.

The uniqueness of this situation in a vertebrate invites study from both a biological and an evolutionary point of view. We have had such an investigation under way for several years. Though our work is still in a preliminary stage, early find-

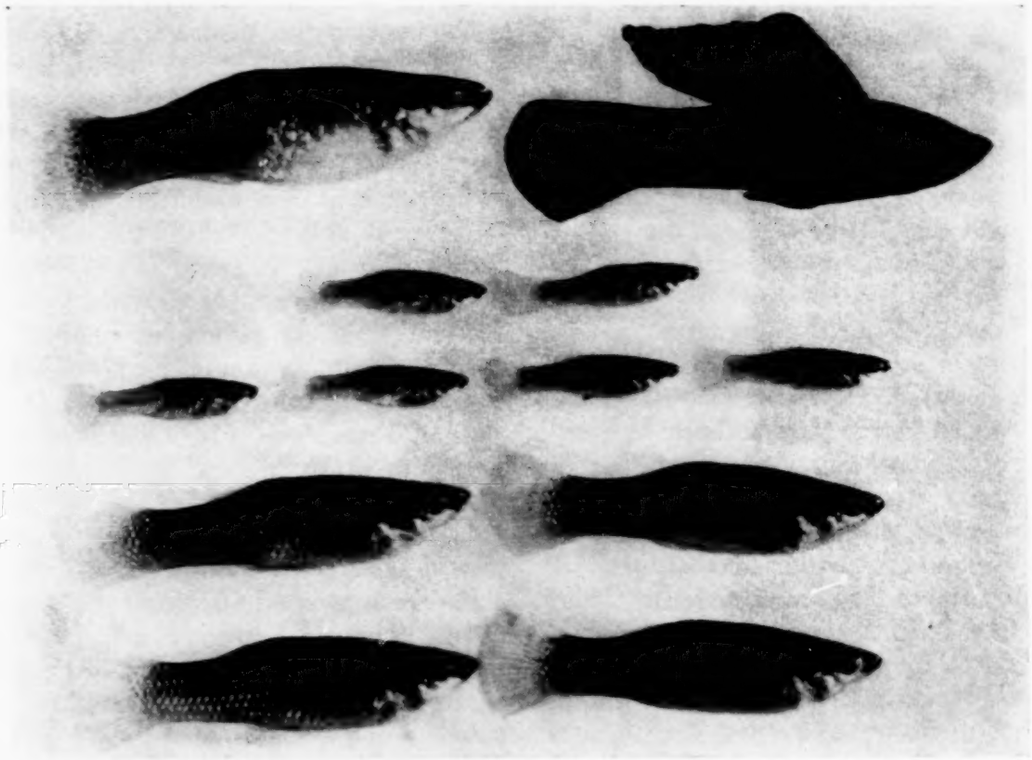

FIG. 1. Upper left, *Mollienisia formosa,* P_1 female. Upper right, *M. latipinna,* P_1 male (melanic form). Lower, F_1 progeny (all female, all *M. formosa*).

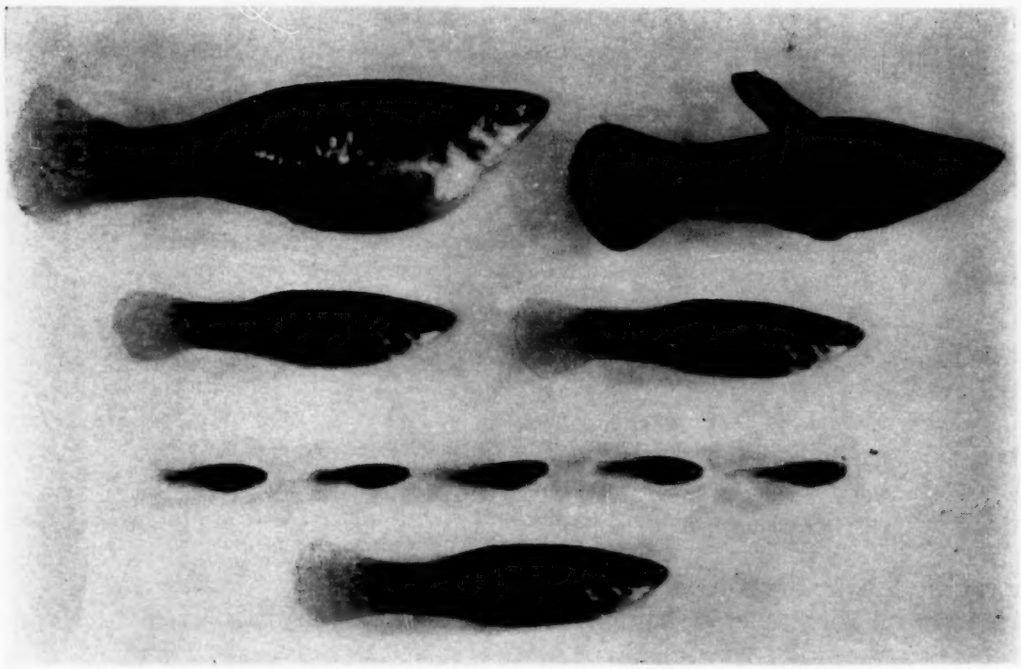

FIG. 2. Upper left, *Mollienisia formosa*, P_1 female. Upper right, *M. sphenops*, P_1 male (melanic form). Lower, F_1 progeny (all female, all *M. formosa*).

ings seem of sufficient general interest to warrant their report at this time.

THE MATERIAL

The original stock of *Mollienisia formosa* was collected in November 1953 from coastal fresh-water pools about Brownsville, Texas. As described by Carl Hubbs, it was found living in close association with a population of *Mollienisia latipinna*. It was brought to the laboratory and carried through several pseudogamic generations using males of the sympatric Brownsville *M. latipinna*. Later a population of *M. latipinna* from eastern Florida was substituted, and the stock has been maintained in mass cultures in large concrete greenhouse pools to the present. Females for use in crossing tests were isolated shortly after birth, and reared in isolation. No controls produced young in the absence of appropriate males.

THE PHENOTYPIC MALE

It seems a reasonable assumption that this curious development and adaptation of pseudogamy in *M. formosa*, with the accompanying loss of males, at least in some populations, occurred at a relatively recent period in the evolution of the species. It then becomes interesting to inquire whether the genes for the specialized characters typical of the male poeciliine (gonopodial structure, secondary sex characters in shape and coloration of fins and body) have been retained in the population, though rarely if ever expressed phenotypically. The situation is very easily investigated by treating females with hormones of the general type of methyl testosterone, well known to have pronounced masculinizing effects in other poeciliid fishes, such as *Platypoecilus maculatus* (Grobstein, 1948) and *Lebistes reticulatus* (Eversole, 1939; Hopper, 1949).

Treatments were carried out following the general procedure of Grobstein in *Platypoecilus* (1948). Crystalline methyl testosterone[1] was dissolved in 95% alcohol to a concentration of 1 mg/ml. A stock solution was prepared by diluting this solution with tap water to a con-

[1] Kindly furnished by the Schering Corporation.

centration of hormone of 0.0035 mg/ml. This dilution was made fresly before use and was added to aquaria containing the treated females twice weekly, to make a final concentration of 0.005 mg/liter. Fish were maintained in fresh aquarium water rather than in the modified Ringer solution described by Grobstein. Treatment ranged from 60 to 90 days.

All females so treated developed the gonopodial structure and body proportions generally typical of males of *Mollienisia,* confirming findings of Carl Hubbs (1955), and a characteristic secondary sex coloration together with a pronounced courtship behavior pattern (fig. 3). The sex color pattern was very uniform in all individuals treated and was distinct from that of males of *M. latipinna* or *M. sphenops.* The many individuals treated gave ample evidence that genetic factors involving the normal array of male secondary sexual characteristics have been maintained intact in the population.

Subsequent to this work, Clark Hubbs, Drewry, and Warburton (1959) discovered a single wild male in the Brownsville population; later, two more were found. The natural occurrence of phenotypic males in these Brownsville populations has thus been demonstrated as a very rare phenomenon, providing suggestive evidence that the genetic factors involved in the normal expression of masculine phenotypic characters have been retained. The three males agree in phenotype with the masculinized females in our own laboratory. It is not yet clear whether they are functional. Tests of this are at present under way by Clark Hubbs and, thanks to his generosity, by us.

One puzzling factor remains, as Clark Hubbs, Drewry, and Warburton have pointed out. These males, together with both Hubbs's and our masculinized females, are phenotypically indistinguishable from F_1 male progeny of the hybrid

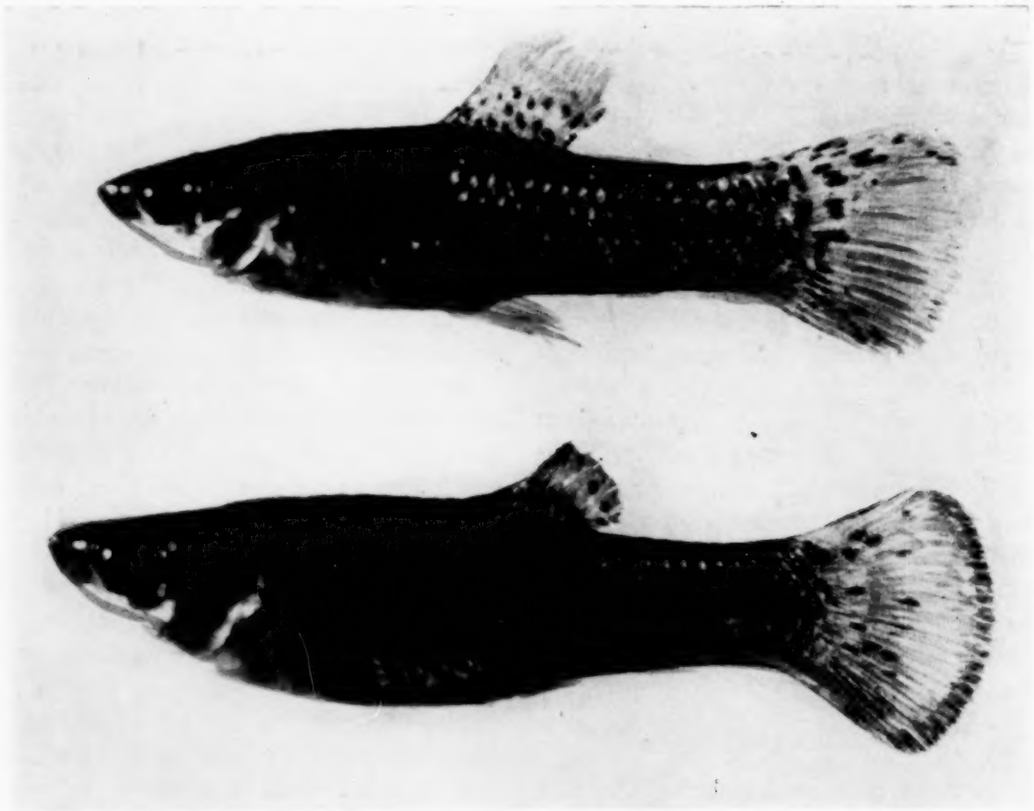

FIG. 3. Upper, *Mollienisia formosa,* female masculinized with methyl testosterone. Lower, *M. formosa,* untreated female.

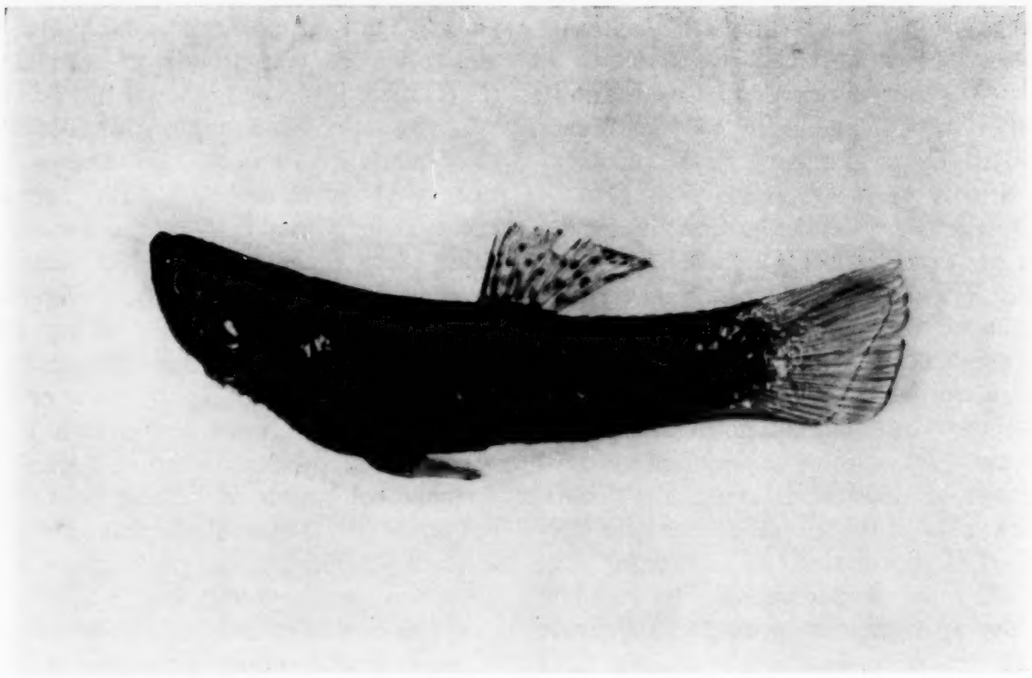

FIG. 4. Male from laboratory (Brownsville) population of *Mollienisia formosa*.

M. latipinna × *M. sphenops*. Thus there is the remote possibility, as the authors suggest, that these males might be naturally occurring F_1 hybrids of the two species. This origin seems unlikely, since the Brownsville population lies far outside the normal range of *M. sphenops*. Although it is possible that *M. sphenops* may have been released occasionally in the area, neither Carl Hubbs, Clark Hubbs, nor we have ever taken such a form in the region in examinations which collectively extend over some twenty years. Hubbs and Hubbs have examined about three thousand individuals; we have examined approximately an additional two thousand. A single aberrant situation, however, must be reported from our own work which bears both on this question and on the general problem of the evolutionary origin of *M. formosa*.

Among a group of some twenty young selected shortly after birth in our laboratory from the Brownsville stock collected in 1953 and reared to maturity in isolation, a single individual, after a period of many months, spontaneously developed masculine characteristics (fig. 4). It proved entirely fertile in matings to *M. formosa*, and more than one hundred F_1 progeny were reared, all normal *M. formosa* females. Carl Hubbs, Clark Hubbs, Robert R. Miller, and we are all of the opinion that this individual was phenotypically a typical male *M. sphenops*. Since all its siblings were normal *M. formosa*, since no adult wild *M. sphenops* has ever been recorded in this population under natural conditions, and since no further such individuals have appeared in this laboratory population over a six-year period, the query naturally arises whether (*a*) introgression of paternal chromosomes or (*b*) abnormal meiotic segregation of maternal chromatin may have been responsible for the production of this individual. The second interpretation accords with the recent suggestion by Clark Hubbs (personal communication) that *M. formosa* may have originated as an allodiploid between species having chromosome counts of $2n = 46$, recorded by Wickbom (1943) for *M. sphenops* and *M. velifera*, or possibly $2n = 44$, recorded for strains of *M. sphenops*. The actual chromosome number of *M. formosa* has

not yet been firmly established. Schultz, however, has obtained two counts of $2n = 46$, and Drewry obtained counts both of $2n = 45$ and of $2n = 46$ (Kallman, 1960). There is strong presumptive evidence that the species is diploid.

On the other hand, the first interpretation has received some experimental support. The possibility of the introgression of genetic elements of *M. latipinna* into the genome of *M. formosa* was demonstrated recently in our laboratory in the following interesting and unexpected manner. A series of experiments is now in progress, designed to repeat the experiments of X-ray sperm inactivation described in the matings of *Ptinus hirtellus* and *P. latro,* and especially to compare the X-ray dosage required to inactivate the sperm of *M. latipinna* in crosses to its own species with that required to inhibit sperm-stimulated gynogenesis in *M. formosa.* For this purpose an all-black race of *M. latipinna* was selected. The black is an extreme form of spotting, producing a uniform heavy melanic pigmentation over the entire body in both sexes. This race has been shown to produce progeny all of which are of this coloration over many generations. In crosses with normal *M. latipinna,* or with normally colored individuals of *M. sphenops* or other species with which *M. latipinna* will produce F_1 progeny sexually, the spotting is reduced, so that the F_1 young show conspicuous heavily melanized patches on the normal background. In many crosses with *M. formosa,* extending over a period of years, all progeny were of typical *M. formosa* coloration, indicating no participation of the paternal chromosomes (at least in regulating final pigment development) in the F_1 young (fig. 1).

Males of this stock of *M. latipinna* were X-rayed and exposed simultaneously to infertile females of their own population and of *M. formosa.* The resulting progeny were readily identifiable as to origin by their coloration.

In all but one case, even at high X-ray

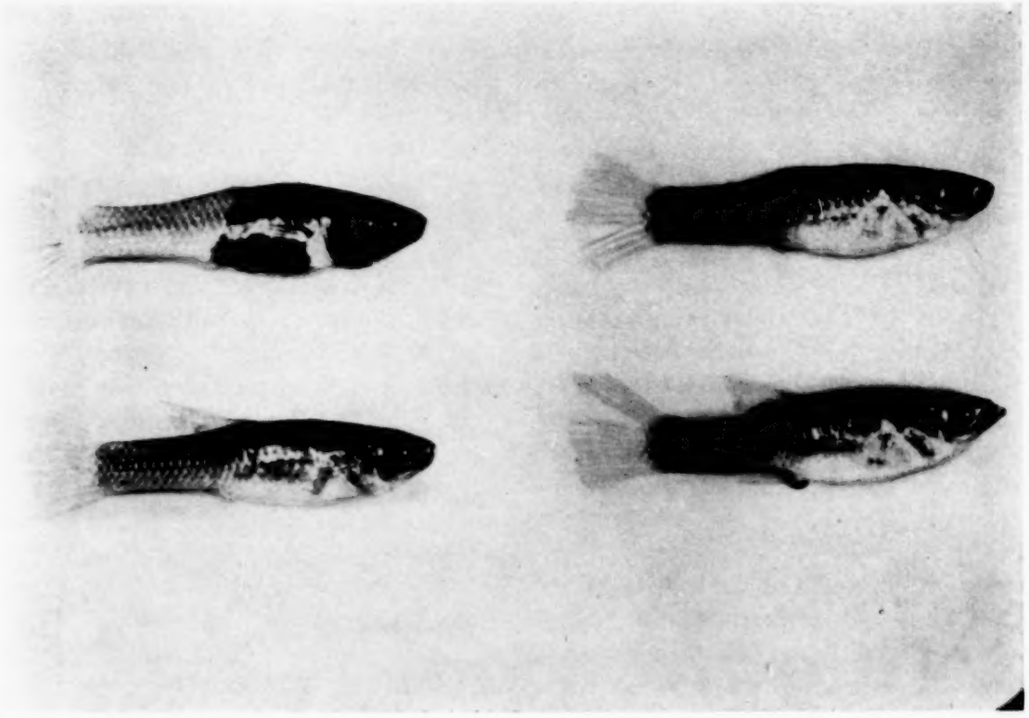

FIG. 5. F_1 progeny: female *Mollienisia formosa* × male *M. latipinna* (melanic). Male x-rayed: 1050 roentgens (250 kv, 30 ma, W target). Upper left: spotted F_1 individual in brood of forty-five; remainder, normal F_1 siblings.

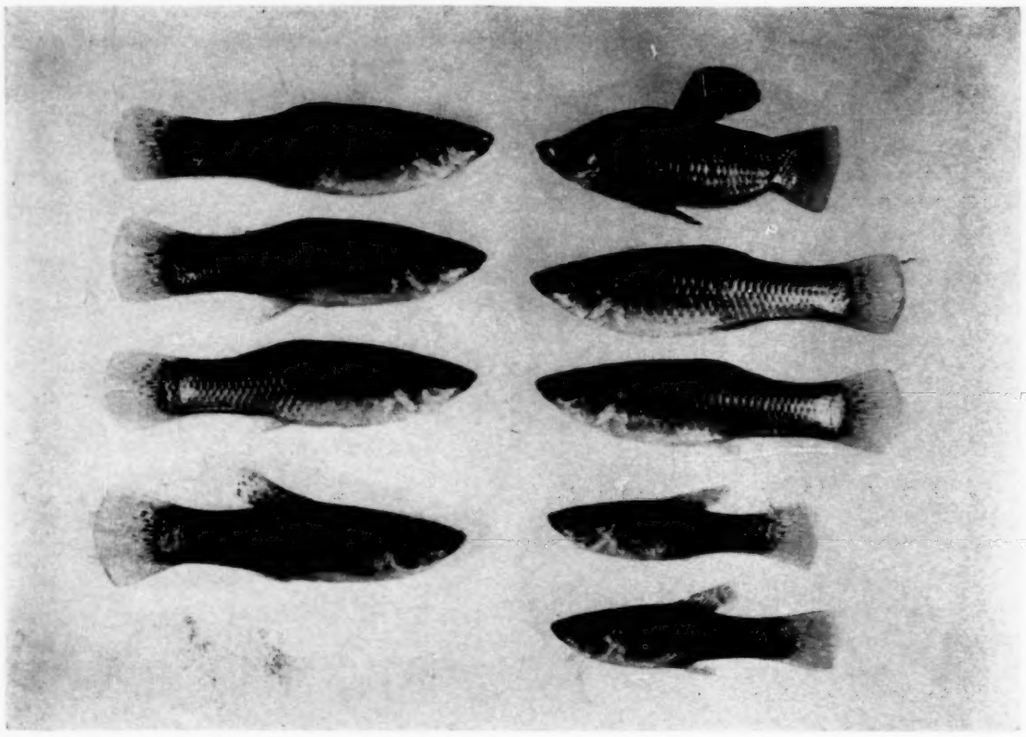

FIG. 6. Upper left, *Mollienisia formosa*, P_1 female. Upper right, *Limia nigrofasciata*, P_1 male. Lower, F_1 progeny (all female, all *M. formosa*).

dosages, the progeny of *M. formosa* females, if they developed at all, were of the normal *M. formosa* coloration. In a single cross, however, the male of which had received a dosage of 1,050 roentgens (standard therapeutic tube, 250 kv, 30 ma, 50-cm target distance, 0.5 mm Cu and 1 mm Al filters) immediately before the cross was made, two heavily spotted young appeared in a single *M. formosa* brood of forty-five individuals. One of these is shown in figure 5. The remaining siblings were normal, as were all those of a further brood produced from the same female and by the same mating. The most plausible interpretation of these two individuals appears to be that they represent rare cases of the influence of paternal *M. latipinna* chromosomes in development.

The matter was of sufficient interest to prompt further investigation using males of various species other than *M. sphenops*, *M. mexicana*, or *M. latipinna*, which are taxonomically related to *M. formosa* but not normally sympatric in range with their populations.

CROSSES WITH RELATED ALLOPATRIC SPECIES

Crosses were made using males of two related genera the ranges of which do not overlap the range of *M. formosa*: *Limia nigrofasciata*, native to the eastern Caribbean, especially Haiti; and *Poecilia vivipara*, with a wide range including Trinidad, coastal Venezuela, the Guianas, and northern Brazil. The males of *L. nigrofasciata* were from an aquarium-bred population obtained commercially in Florida. The males of *P. vivipara* were from a wild population collected in Trinidad. In both crosses the results were as with *M. latipinna* and *M. sphenops*, all-female broods being produced pseudogamically, with no trace of introgression of the characteristics of the male species (fig. 6). The matter was further tested by treating some of the F_1 females with methyl tes-

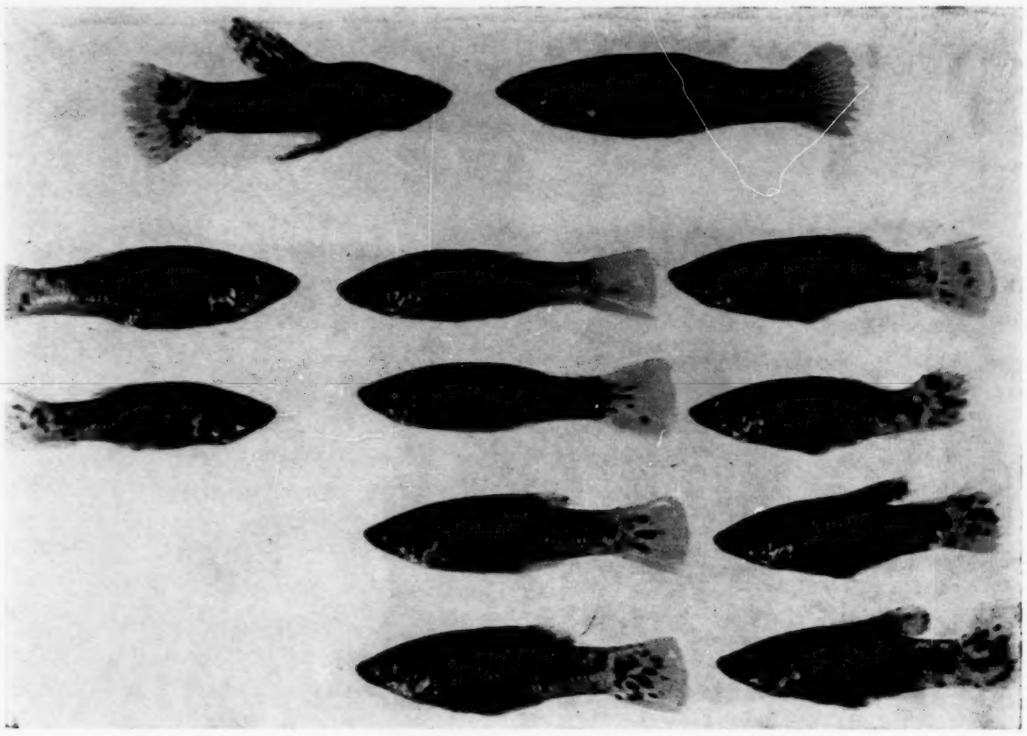

FIG. 7. Upper right, *Mollienisia formosa*, P_1 female. Upper left, *Limia vittata*, P_1 male. Lower, F_1 progeny (left row, females; center and right rows, males).

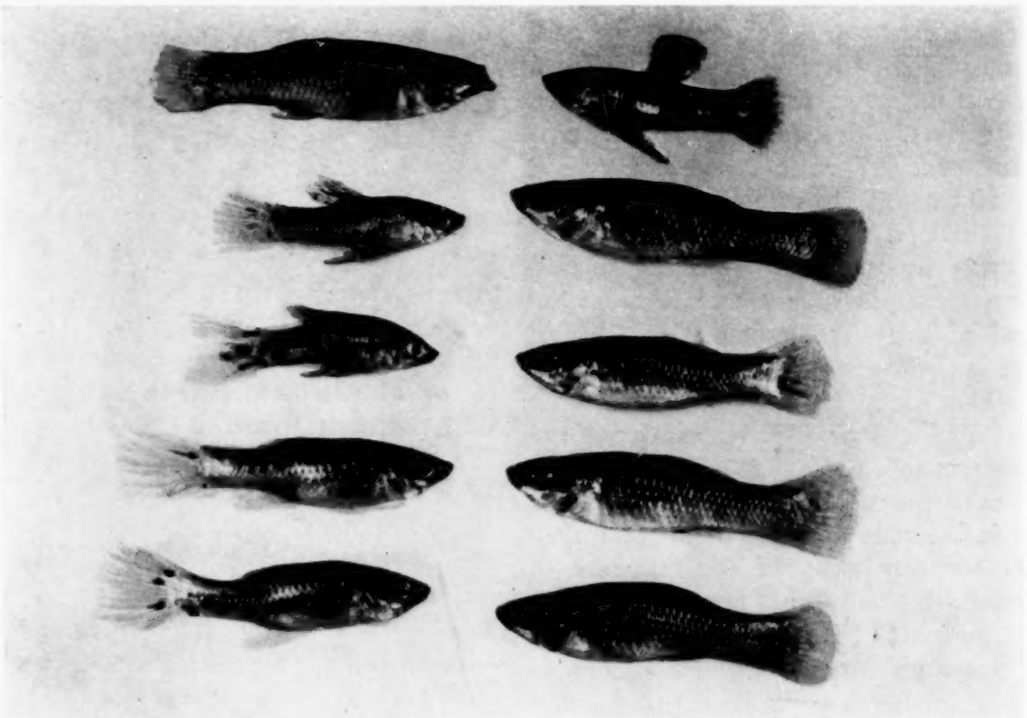

FIG. 8. Upper left, *Mollienisia formosa*, P_1 female. Upper right, male, F_1 hybrid (male *Limia vittata* × female *M. formosa*). Lower, backcross progeny (upper left, males *L. vittata*-type; lower left, females *L. vittata*-type; right, females *M. formosa*).

tosterone. They developed the male coloration typical of *M. formosa.*

Sexual Reproduction in *Mollienisia formosa*

A more surprising result, however, was obtained when crosses were made between females of *M. formosa* and males of *Limia vittata.* The normal range of *L. vittata* is in the eastern Caribbean, especially Cuba. A heavily spotted aquarium-derived population, obtained commercially in New York City, was used as the source of males, in order to provide suitable phenotypic markers.

Five crosses of these males to females of *M. formosa* were made. In each cross the F_1 young developed into forms phenotypically indistinguishable from *L. vittata* both in form and in coloration (fig. 7). They proved to be predominantly males, but, in one group of eighteen, three females were produced; in a group of twenty-four, there was a single female. F_1 males and females proved fully fertile in crosses *inter se,* the F_2 progeny (and the subsequent mass culture) proving indistinguishable from *L. vittata.* Males also proved fully fertile when backcrossed to females of *L. vittata.*

Most interesting results were obtained when F_1 males were backcrossed to females of *M. formosa* (fig. 8). Mixed broods resulted, the evident consequence of competition between sexual and pseudogamic reproduction in a single lot of eggs fertilized by the sperm from a single male. In the first generation of such a cross, gynogenesis predominated. When the group was maintained in mass culture for several months in large concrete pools, sexual reproduction outran pseudogamy,

Table 1

	M. formosa		*L. vittata*-like hybrids		
Date	Females	Young	Females	Males	Young
3/2/57	56	37	14	7	30
8/23/57	61	Not recorded		34	270*

*Adult females and all young taken together.

Table 2. *Frequencies of ray numbers in dorsal and caudal fins in smaples of* M. formosa, L. vittata, *and* F_1 *progeny*

No. dorsal rays	*M. formosa*	*L. vittata*	F_1 progeny
10	1	7	18
11	2	0	7
12	13	0	0
No. in total samples	16	7	25
No. caudal rays			
25	0	0	1
26	0	0	0
27	0	0	4
28	2	0	12
29	0	5	8
30	4	1	0
31	10	1	0
No. in total samples	16	7	25

leading to a predominance of *L. vittata*-like forms, as indicated in table 1 and table 2.

Discussion

Many fascinating evolutionary problems are raised by this example of sperm-stimulated parthenogeny as a normal reproductive device, apparently unique among vertebrates. What, for example, is the precise range of the species in relation to its host forms? How recently in its evolution has sexuality been abandoned as the normal reproductive mode? How important is sexual introgression of "host"-species characteristics in the evolution of the species today? Has significant evolution, indeed, occurred since the loss of sexuality?

Further work must be done before these questions can be adequately answered. Yet there are bits of suggestive evidence, mere tips of the iceberg, in respect to several of them. The retention of a complete potentiality for the development of male secondary sexual characteristics in apparently all females, readily evoked in the laboratory with methyl testosterone, suggests that the modification to pseudogamy has taken place late in the evolution of the species and probably rather recently in time. Again, the results reported here suggest that introgression of paternal characteristics from one of the "host" species may not be so rare an

occurrence as to be without evolutionary significance.

Finally, Kallman (1960) has separately reported some extremely interesting experiments in tissue transplantation undertaken with this same stock of *M. formosa* which suggest that within this one restricted wild population, the members of which are morphologically indistinguishable, several quite distinct clones may coexist showing mutual tissue incompatibility—presumably reflecting the occurrence of tissue-compatibility mutations after the assumption of pseudogamy as the normal reproductive mode in the population.

The fineness of the distinction between sexual and pseudogamic reproduction in *M. formosa* (in a physiological sense) is suggested by the mixed sexual and parthenogenetic broods which result when the F_1 hybrid males of the *M. formosa* × *L. vittata* cross are backcrossed to *M. formosa*. It is even more pointedly emphasized by the fact, recently ascertained, that sexual reproduction of the kind described may not occur in all populations of *L. vittata*, or in all females of this population of *M. formosa*. Thus two crosses of *M. formosa* females from this population with *L. vittata* males from a population commercially obtained from the west coast of Florida have yielded 115 F_1 young, all proving to be *M. formosa* females pseudogamically produced and thus corresponding to the results with *L. nigrofasciata*, *P. vivipara*, and the two species of *Mollienisia*.

It is clear that much remains to be learned concerning the evolution of this singular poeciliid species.

Summary

Preliminary findings are given in an investigation currently in progress of naturally occurring pseudogamous reproduction in the viviparous poeciliid fish *Mollienisia formosa*. Results obtained with crosses of this "all-female" form with the sympatric and normally bisexual species *M. latipinna* and *M. sphenops* confirm those of earlier workers: *M. formosa* females exhibited exclusively thelyotokous parthenogeny under sperm stimulus of these species, except in one interesting case, here reported, where introgression of a paternal characteristic from *M. latipinna* seems to have occurred. Results obtained in crosses to males of two more distantly related and allopatric forms, *Limia nigrofasciata* and *Poecilia vivipara*, showed similar apparent gynogenesis.

With one race of *Limia vittata*, however, sexual reproduction proved the rule. The bisexual F_1 progeny showed phenotypic similarity to and complete fertility with the male *L. vittata* parent. Males markedly predominated in the F_1 progeny. Backcrosses of the F_1 males to *M. formosa* resulted in mixed broods, with parthenogenetic females predominating in the first generation but rapidly being exceeded in numbers by their sexual siblings and their sexual descendants within three generations in mass culture.

A fertile male, apparently phenotypically *M. sphenops*, developed among the isolated laboratory descendants (otherwise all *M. formosa* females) of individuals originally taken from a wild population of *M. formosa*. It is described and discussed in the context of the recent discovery of apparently naturally occurring males of *M. formosa* by Hubbs, Drewry, and Warburton.

The possible evolutionary significance of these findings, and of those of Kallman on tissue transplantation within the species, is briefly considered.

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ISOLATING MECHANISMS IN THREE SYMPATRIC TREEFROGS IN THE CANAL ZONE[1]

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Three species of small treefrogs, *Hyla microcephala* Cope, *H. phlebodes* Stejneger, and *H. ebraccata* Cope, are sympatric in the Canal Zone. They are rather similar in morphological appearance, and have similar breeding calls, and commonly call and breed in the same areas during the same periods of time. The question arises: how are these three frogs able to maintain their identities as discrete species under these conditions?

Investigations were carried out in the Canal Zone during the period from April 1955 to November 1956, while stationed there with the U. S. Air Force, and during the summer months of 1958. The area studied was in the lowlands of the Pacific drainage of the Canal Zone, chiefly within a four-mile radius of the town of Pedro Miguel.

All three species are of rather small body size (mean snout-vent length, millimeters—*ebraccata:* males 24.6, females 31.2; *microcephala:* males 22.8, females 27.1; *phlebodes:* males 22.2, females 27.4), and are quite similar in form and proportions. The basic color of all three, when active in the field (at night) is generally a rather vivid yellow with brown markings. The chief noticeable difference is in pattern, and in this characteristic *ebraccata* is the most distinctive. More detailed comparisons of the morphology will be presented elsewhere. Suffice it to say that the data from this source suggest that the species are quite closely related.

The scheme of classification used as a guide in checking the possible types of isolating mechanisms is presented below. It is designed basically after the concept of Muller (1942) and represents an attempt to combine the best ideas of other classifications to apply to the problems of anuran speciation.

I. Anti-mating isolating mechanisms
  A. Geographic isolation (allopatry) (Spatial Isolation)

  (Reproductive Isolation)
  B. Habitat isolation (difference in breeding site)
  C. Seasonal isolation (difference in breeding season)
  D. Temporal isolation (difference in time of day breeding occurs)
  E. Psychological or Climatic isolation (difference in response to climatic and similar physical environmental factors)

II. Courtship isolating mechanisms
  F. Ethological isolation (difference in courtship behavior)
  G. Mechanical isolation (structural difference preventing interbreeding)

III. Post-mating isolating mechanisms
  H. Gametic isolation (incompatibility of gametes)
  I. Hybrid inviability
  J. Hybrid sterility
  K. Hybrid selective inferiority.

Anti-mating mechanisms, a category more or less equivalent to Muller's "bars to crossing," are factors which do not involve a wastage of gametes or other waste of reproductive energy resulting from attempts to breed with the wrong species. The term "anti-mating" is used in preference to Muller's term only because it combines well with "mechanisms," and is

[1] Part of a dissertation submitted to the University of Texas in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

preferred to "pre-mating" (Littlejohn, 1957, 1959; Mecham, in press) because the latter term carries an implication that mating will occur.

Courtship mechanisms, an intermediate grouping, may sometimes allow a delay in breeding with the correct species, or even wastage of gametes, but at other times may not. These are all mechanisms which involve reactions between individuals, whereas the first category includes mechanisms which involve reactions between an individual and the physical environment, and the division following includes mechanisms which involve reactions between gametes (or various stages of resulting zygotes).

Post-mating mechanisms are those which are characterized by wastage of gametes. This is equivalent to Muller's "incapacitation of hybrids," and is used in the same way that Littlejohn and Mecham have used the term.

The simpler divisions are much the same as in Gulick's (1890) original classification, as modified, chiefly in terminology, by Romanes (1906), Robson (1928), Robson and Richards (1936), Dobzhansky (1937) and others.

Anti-Mating Mechanisms

The treefrogs were located by tracing the sound of their breeding choruses. The general ecology of these calling stations was noted with reference to (1) type of vegetation; (2) distribution of calling frogs in relation to water and vegetation, as well as in relation to other treefrogs of the same and other species; (3) temperature of the air and water; (4) weather conditions; and (5) local time.

Habitat isolation. The three species appear to differ somewhat in their preference for breeding sites, but their tolerances overlap to a large extent. *H. microcephala* apparently breeds in any area of standing water which has grass or sedge growing in it. If the grass is tall males generally call from a position just above the water, or just above the ground near the water. These frogs commonly call in roadside ditches, boggy areas of second growth where grasses are abundant, and grassy fields in low spots where water is standing. *H. phlebodes* apparently prefers a breeding site of dense, rather high grasses, usually in bogs or swamps, but may also breed in roadside ditches. *H. ebraccata* also seems to prefer high vegetation and rather permanent standing water. Males frequently call from roadside ditches, generally from a tangle of vines and woody vegetation, or from thick clumps of high grass somewhat up and away from the ditch proper. In contrast, when *microcephala* calls from a ditch it usually does so from grass growing in the water, while *phlebodes* may call from either position but is usually up away from the water, and prefers the high grass to the vines and woody vegetation.

Because of the overlap of breeding-site tolerances, such isolation as is effected by the differences in preference breaks down in many places, and all three species are commonly found calling at the same location. I have often observed individuals of all three species calling within a few inches of one another. Sometimes there was a concentration of individuals of the same species in one part of the area and another species in another part, and at other times there was apparently random intermingling of the species. More often, one of the species was dominant in number of calling individuals and was dispersed throughout the area, while the other two were intermingled with the dominant species, but more or less concentrated in different parts of the area.

A rather careful study of calling stations was made at a swamp about seven miles north of Miraflores Locks, where the three species commonly call together. All three generally call from the tall scandent grass which grows in the water, and no significant difference in calling station could be detected. Another aspect of the breeding habitat is the egg-deposition site, and a difference was found in this factor for two of the species. A

pair of *microcephala* was observed spawning, and the eggs were deposited in small masses on the surface of the water. A small mass of *ebraccata* eggs was found clinging to a blade of grass about 20 inches above the water. Taylor (1951) also reported that *ebraccata* lays its eggs on vegetation above the water. This utilization of different parts of the habitat for oviposition may support reproductive isolation to some extent. The ovipositional habits of *phlebodes* are unknown.

Seasonal isolation. The three species seem to call strongly throughout the rainy season, which is generally from mid-May to late December or early January. During the dry season, *microcephala* apparently migrates to permanent lakes and sloughs formed by waters backed up from the Canal, and continues to call from floating vegetation in the water. There is apparently no breeding in the dry season, although this has not been thoroughly investigated. Periods during which one of the species forms large choruses to the extent that it is the dominant species calling in many localities are from about May to November for *ebraccata,* May to January for *microcephala,* and May to September for *phlebodes.* Breeding peaks (based primarily on presence of amplexing pairs, and secondarily on increased calling activity) are apparently June–July and possibly November for *ebraccata,* May–July and October for *microcephala,* and May–September for *phlebodes.* Thus the breeding season of these three species overlaps completely, although there may be some isolation due to differences in the actual breeding peaks.

Temporal isolation. Little difference was found in the time of day during which mating occurs in the the three species. The dominant calling period is about the same in all three, from about sunset until shortly after midnight. The earliest that *ebraccata* and *phlebodes* were heard calling strongly was 7 P.M., and *microcephala* as early as 6:45 P.M. The latest that *ebraccata* and *phlebodes* were heard calling strongly was midnight, and *microcephala* as late as 1:45 A.M. Occasional calls of all three species were heard beyond this period, both earlier and later, but they were not calling *strongly,* i.e., repeating their calls at regular intervals and calling in choruses rather than as scattered individuals.

The earliest that actual breeding was observed was about the same for all, between 8 and 9 P.M. The latest time amplexing pairs of *microcephala* were observed (1:30 A.M.) is significantly later than for the others (9:30 for *phlebodes,* 10:20 for *ebraccata*), but probably only because of the much greater abundance of *microcephala,* making observation of amplexing pairs of that species more likely at all periods. Thus there is probably no daily temporal factor of any significance contributing to the reproductive isolation of these species.

Psychological or climatic isolation. Temperature data indicate that there is no correlation between this factor and breeding activity of any of the species. All were found calling strongly and breeding throughout the entire range of nocturnal air temperatures measured during the breeding season (22°–28° C). It probably does not normally get too cool or too warm for any of the species to call and breed during the breeding season, which is more or less to be expected in a tropical area. Also the light of the moon apparently had no effect on the breeding and calling activity of the three forms. The number of individuals calling and clasping apparently was independent of whether the night was dark and moonless or brightly lit by a full moon, or any intermediate condition.

There is no apparent correlation of rainfall with the activities of *ebraccata;* however, there seems to be a tendency for *microcephala* to increase its activity immediately after heavy rains, whereas *phlebodes* seems to show increased activity after a few days of little or no precipitation. This is based on generalized observations and cannot be supported by

definite data. Evidence that this tendency is real seems to be supported by a decrease in activity of *phlebodes* in October and November, when the rains are heaviest and most frequent, and by a breeding peak in *microcephala* during October. Thus among the psychological factors checked, only a possible subtle difference in response to rainfall between two of the species may have some bearing in supporting reproductive isolation.

Courtship Mechanisms

Ethological isolation. The most significant factor which may serve to isolate species of frogs appears to be the breeding call. It is generally accepted that the breeding call of male frogs serves at least two important functions. First of all, it is the sexual "display" which attracts the female. Several workers have reported observing females of various species approaching calling males of their own species. Among frogs of the family Hylidae (Blair, 1958; Littlejohn, 1958; Mecham, in press), the male usually continues calling, apparently oblivious to the presence of the female until she makes physical contact, at which time the male stops calling and amplexus occurs. In addition, Martof and Thompson (1958) recently demonstrated experimentally that female *Pseudacris triseriata feriarum* are attracted to the call of the male of that species.

The other important function generally attributed to the call is that it is the primary method of species recognition. Until recently, most workers tended to accept this hypothesis with no experimental evidence to support it. However, Littlejohn and Michaud (1959) demonstrated that female *Pseudacris streckeri* respond to the call of the male of that species, but not to the call of the sympatric species *P. clarki*, thus presenting conclusive experimental evidence that the female frogs can discriminate between the two calls. This establishes the fact that difference in breeding call may act as an isolating mechanism in frogs. Blair and Littlejohn (1960) further showed that female *Pseudacris streckeri* effectively discriminate in favor of the call of male *streckeri* when given the choice between this and the call of the closely related allopatric *P. ornata,* which has a call that apparently differs from *streckeri* only by about a 20% average difference in dominant frequency. The two calls are so similar that it is very unlikely that a human ear could distinguish them, so that the discriminatory ability of some frogs may be extremely well developed.

The breeding calls of individual male treefrogs of all three species were recorded in the Canal Zone using a Magnemite (Model W610EV) battery-operated tape recorder, at a tape-speed of 15 inches per second, and a Shure dynamic microphone (Model 55S). The calls were later analyzed in the laboratory at the University of Texas by means of a Kay Electric Co. Sona-Graph. Blair and Pettus (1954), Blair (1955), and Fouquette (in press) have described most phases of this method in detail. Sample audio-spectrograms (sonagrams) of normal and abnormal calls of all three species are shown in figures 1 and 2. The frogs were usually collected and preserved so as to permit correlation of any given call with the individual that produced it. Temperatures were recorded but it was found that there was so little variation in this factor here that it could be disregarded in comparing the calls.

The breeding calls of the three frogs are rather similar, and it is believed that this is a reflection of their close relationship. However, they are readily distinguished by the human ear, with a little practice, so it is assumed that they are distinguishable to the frog's ear. Data will be presented to demonstrate good quantitative differences in the three calls, thus providing a basis for ethological isolation.

The calls of the three species consist of a series of short buzzes or squeaks. Each of these short bursts of sound is

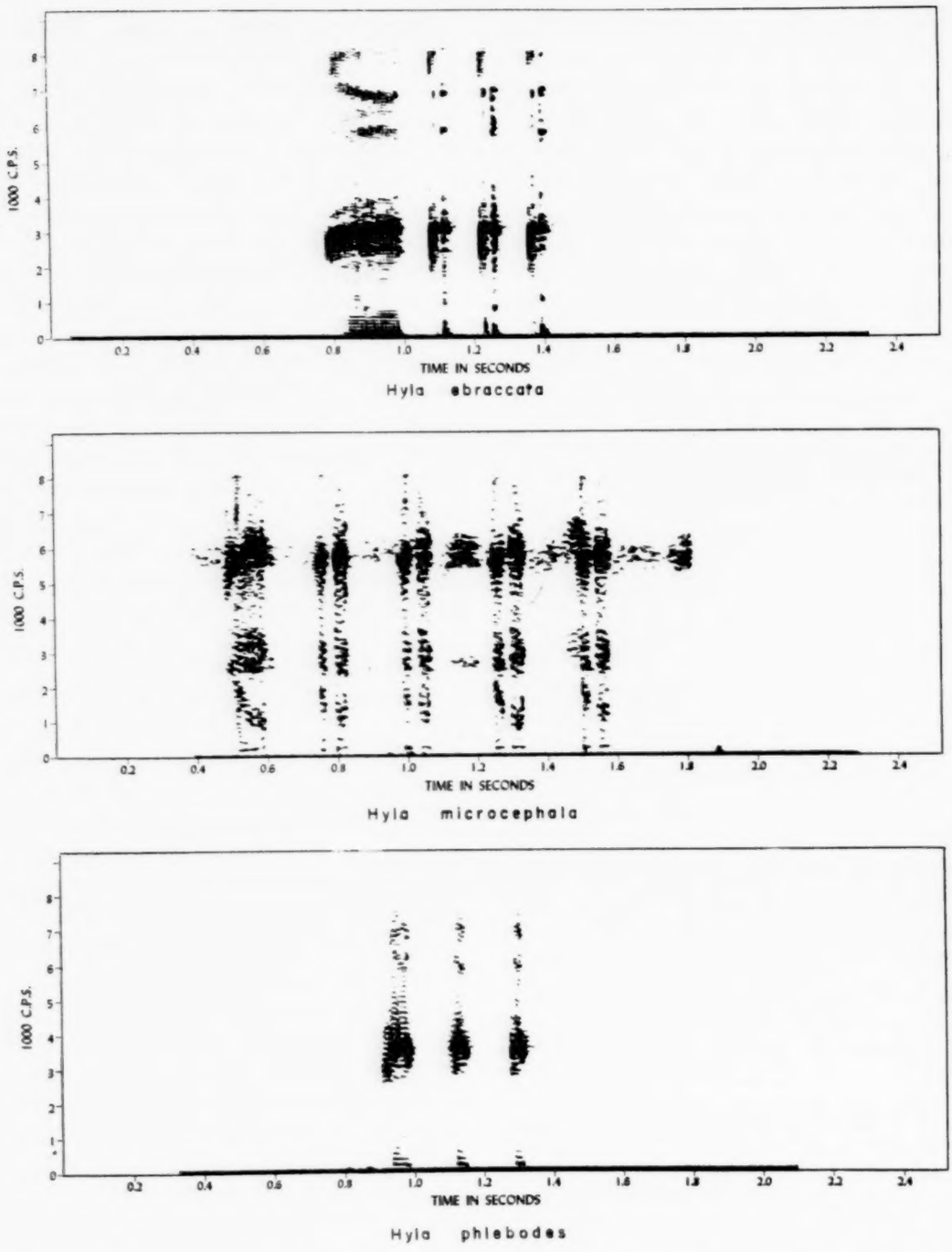

FIG. 1. Sonagrams of normal calls of three species of *Hyla*, recorded in the Canal Zone. For each species is shown a representative call-group composed of a single primary note followed by several secondary notes.

termed a *note*. Characteristically, the call pattern consists of a group of several of these notes repeated in rapid succession. This is followed by a longer pause, which is followed in turn by another group of notes, and so on. Each of these groups of notes is referred to as a *call-group*. The first note of a call-group is longer in duration than those that follow it and usually differs somewhat in frequency characteristics. Consequently, it is distinguished from others as the *pri-*

mary note, while the others are called ***secondary notes.*** A call-group may consist of one note (always a primary note) or, in the sample studied, up to 29 notes (one primary and 28 secondaries). Call-groups with more than one note are referred to as *compound* call-groups.

The *fundamental* frequency is the fre-

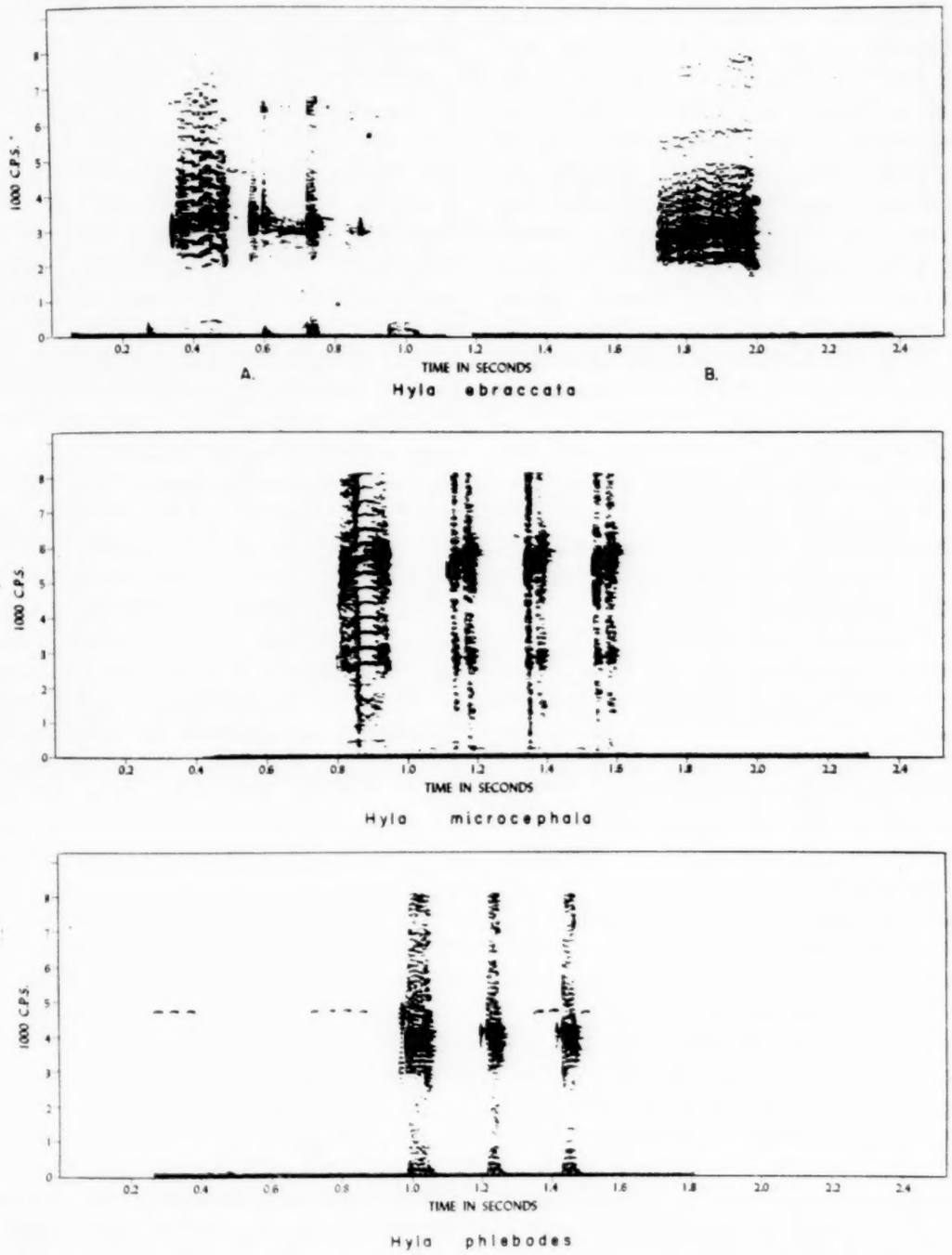

FIG. 2. Sonagrams of abnormal calls of three species of *Hyla. H. ebraccata:* Call-group to the left composed of a *high primary* note, and a normal *paired secondary* note and an *unpaired secondary* note. Call-group to the right consists of a single *high primary* note. *H. microcephala:* Call-group composed of a *high primary* note and three normal paired secondary notes. *H. phlebodes:* Call-group in which each note has a short auxiliary component preceding it. Such notes are considered as *paired* (see text).

quency of vibration of the vocal cords and is very important in determining the way a call "sounds." This characteristic was measured to the nearest cycle per second, but the accuracy is probably not this great. The *dominant* frequency is the frequency resonated by the vocal sac of the frog. It is chiefly a function of the size of the vocal sac and is not correlated with the fundamental. The fundamental may be drastically changed without causing any significant change in the dominant frequency. However, when such a change in the fundamental occurs, there is a very noticeable change in the "sound" of the call, in both the pitch and quality. Probably it is the fundamental and dominant together which are chiefly responsible for the "sound" of a call.

In a simple call, the dominant frequency is a harmonic of the fundamental. However, in the calls of the three *Hyla* species studied here, a dominant frequency band cuts across the harmonics of the fundamental, obscuring the harmonic pattern, and shifting upward in freqency throughout the duration of the note. Arbitrarily, I have selected the mid-point of this band measured at the terminal border of the note, and measured this for each note to the nearest 50 cps. This will be referred to henceforth as the *mid-point of the dominant* or simply as the *dominant*.

The *duration* of each note was measured to the nearest .01 second, and an average of three or more notes per individual rounded off to the nearest .005 second. The *note-repetition rate* was also measured. This is the rate, in notes per minute, at which the notes of a call-group are produced. This is applicable only to compound groups, of course. Other characteristics of the calls were also noted and all of these are included in table 1. Among these other characteristics are the approximate areas of the emphasized harmonics other than the dominant and fundamental. These are merely estimates based on one or two measurements and are presented merely to help fill in the structure of the call. The number of notes in each call-group recorded was counted, and the mean number computed for each individual. The over-all range in this number is given for each species, as well as a mean calculated from the individual means.

H. ebraccata has the lowest pitched call of the three and to my ear the loudest. The low pitch is attributable to the fact that it has the lowest fundamental and dominant frequencies. Because the fundamental is of such a low frequency it imparts to the call an almost "trilled" quality. That is, the human ear can almost discriminate the individual modulations. The rather long primary note is usually followed by one to five very short secondary notes. The secondary notes have a slightly higher fundamental, and a somewhat lower dominant frequency than the primary note. The note-repetition rate is generally a little faster than that of the other species. The secondary note is actually composed of two notes which are so closely spaced that it is not usually noticeable to the human ear. This type of note will be referred to as a *paired secondary*, as opposed to an *unpaired secondary* which has a single component. Over half of the individuals recorded would occasionally emit a primary note which sounded high-pitched and squeaky. This type of note is referred to as a *high primary*. Upon analysis it is found that the only major change is a drastice rise in the fundamental frequency (figure 2). The dominant is scarcely affected, although it is usually slightly lower than that of the normal primary.

The call of *microcephala* is very similar in over-all pattern to that of *ebraccata*. The primary note is generally of shorter duration and the secondary note of longer duration than the comparable notes of *ebraccata*. Of the 40 individuals recorded, only one had all unpaired secondary notes, and only two others had an occasional unpaired note. This species has the highest fundamental, the highest dominant frequency, the slowest note-

TABLE 1. *Characteristics of the breeding calls. P(n) = normal primary note, P(hi) = high primary, S = secondary note. Standard deviation is given, rather than standard error, since the dispersion is of more interest than the level of significance in regard to call as an isolating mechanism*

		ebraccata			*microcephala*			*phlebodes*	
		P(n)	P(hi)	S	P(n)	P(hi)	S	P(n)	S
Mid-point dominant frequency (cps)	Mean ±std. dev.	3,064 ±58.9	3,060 ±208.2	3,014 ±181.9	5,636 ±193.8	5,570 ±175.6	5,540 ±148.9	3,580 ±256.7	3,557 ±220.7
	Absolute range	2,500–3,450	2,600–3,300	2,500–3,250	4,850–6,000	5,300–5,800	4,850–5,900	2,450–4,100	2,400–4,100
	Rng. indiv. avgs.	2,630–3,367	2,600–3,300	2,583–3,225	5,150–5,962	5,300–5,800	5,262–5,886	2,800–4,067	2,900–4,050
	Sample size	19	10	19	40	11	39	26	24
Fundamental frequency (cps)	Mean ±std. dev.	87.9 ±6.0	357.4 ±64.6	98.8 ±9.7	206.9 ±13.0	385.1 ±30.8	224.5 ±13.3	149.2 ±6.7	154.9 ±4.7
	Absolute range	75–92	251–436	78–114	184–238	315–419	200–254	122–158	145–162
	Sample size	19	10	18	38	11	21	26	23
Areas of other emphasized harmonies (approx.)		175			2,400			300	
		6,000	—	as P(n)	8,500	—	as P(n)	7,000	as P(n)
		9,000			11,000			10,500	
		12,000			13,500			14,000	
Duration (seconds)	Mean ±std. dev.	.210 ±.025	.180 ±.046	.068 ±.012	.133 ±.010	.143 ±.020	.106 ±.003	.112 ±.018	.082 ±.013
	Absolute range	.16–.29	.15–.30	.04–.10	.08–.17	.11–.18	.08–.14	.07–.16	.05–.12
	Rng. indiv. avgs.	.18–.275	.15–.30	.04–.09	.10–.155	11.–.18	.09–.135	.085–.14	.055–.11
	Sample size	19	10	19	40	11	38	26	24
Notes per group	Mean indiv. avgs.		1.9			3.4		2.3	
	Absolute range		1–6			1–19		1–29	
% groups compound (combining all calls of all individuals recorded)			53.9%			62.0%		32.0%	
Note repetition rate (compound groups)	Mean		351.6			257.8		290.1	
	Absolute range		217–429 (notes per minute)			192–311		206–348	
% Primary notes with high fundamental (all calls combined)			15.0%			3.5%		0.00%	
% individuals recorded with high fundamental			52.6%			27.5%		0.00%	
% Primary notes with high fundamental in individuals that demonstrate it			21.8%			14.6%		—	

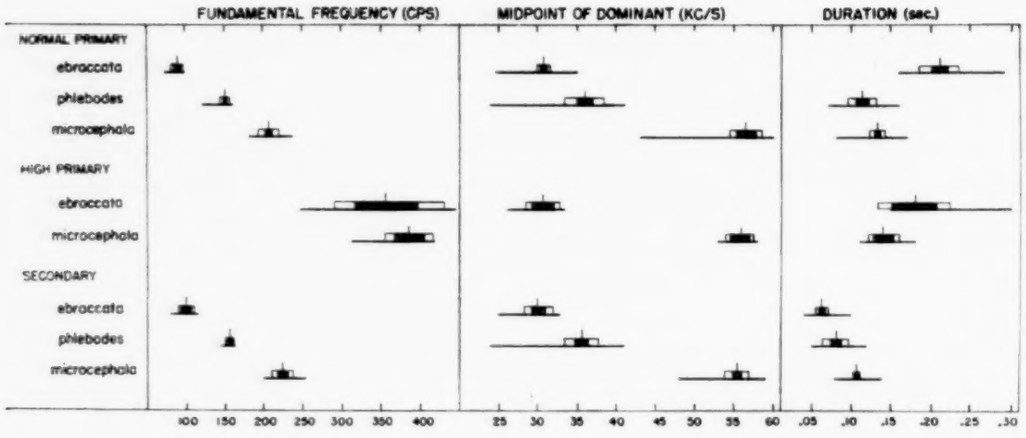

FIG. 3. Comparative call structure. The bar graphs follow the method of Hubbs and Hubbs (1953).

repetition rate, and generally the most notes per call-group of the three species. As in *ebraccata* some of the *microcephala* occasionally emit a high primary note (fig. 2). In both species the primary is the only note which undergoes this phenomenon. The occurrence of this high primary note is not as common in *microcephala* as it is in *ebraccata,* since only 27.5% of the individuals recorded demonstrated it.

The call of *phlebodes* is intermediate between the calls of the other species in most characteristics, including the fundamental and dominant frequencies. Only about one-third of the call-groups are compound, but when the group is composed of more than one note, the number of notes per group is often very high, as many as 29 in one individual. The secondary notes differ from the primary in that they are of shorter duration, but in other characteristics the two are essentially similar, although the secondaries generally tend to have a slightly higher fundamental and a lower dominant frequency than the primary. In *phlebodes* the secondary note is almost always unpaired, although in three individuals there is an auxiliary component with some of the notes, including the primaries, so that it gives the same impression audibly as the paired notes of the other species. This is not structurally the same type of double-note found in the other species (fig. 2), but since it sounds similar to my ear it is considered as a paired note for the purposes of comparisons with the other species in figure 4. A high primary note is not known in this species.

The differences in fundamental frequency among the three species are highly significant (fig. 3), with no overlap in either normal primary or secondary notes. The high primary notes of *microcephala* and *ebraccata* have very erratic fundamental frequencies, and the two overlap broadly. The difference between the means ($\pm$ standard error) of the normal primary notes of *ebraccata* and *phlebodes* is 61.3 ± 1.91 cps, between those of *phlebodes* and *microcephala* it is 57.7 ± 2.47 cps, and between those of *ebraccata* and *microcephala* it is 119.0 ± 2.52 cps. The differences in the secondary notes are of the same magnitudes. The difference between the means of the high primary notes of *ebraccata* and *microcephala* is 27.7 ± 22.43 cps, which is not significant.

The dominant frequencies are significantly different in all three species, although there is considerable overlap in this character between *ebraccata* and *phlebodes* (fig. 3). The difference between the means of the normal primary notes is 516 ± 51.2 cps. There is no overlap in the range of dominant frequencies between *microcephala* and the other species. The difference between the means of *microcephala* and *phlebodes* is

2,056 ± 59.0 cps, and between those of *microcephala* and *ebraccata* it is 2,572 ± 33.4 cps. The differences between the means of the dominant frequencies of both the high-primary and secondary notes are of the same magnitude.

The durations of normal primary and secondary notes overlap considerably, although the means differ significantly (fig. 3). The difference between the means of the normal primary notes of *phlebodes* and *microcephala* is .021 ± .0037 seconds, between those of *microcephala* and *ebraccata* is .077 ± .0065 seconds, and between those of *phlebodes* and *ebraccata* is .098 ± .0042 seconds. The difference between the means of the durations of the high primary notes of *ebraccata* and *microcephala* is .037 ± .0157 seconds, which is of rather low significance. The difference between the means of the secondary notes of *ebraccata* and *phlebodes* is .01 ± .0039 seconds, between those of *phlebodes* and *microcephala* is .024 ± .0025 seconds, and between those of *ebraccata* and *microcephala* is .038 ± .0030 seconds.

In using the various measurements of call structure as taxonomic characters we observe that there are highly significant differences in most of those structural characteristics analyzed. However, the effectiveness of call as an isolating mechanism depends upon virtual complete lack of overlap in the characteristics which the females discriminate. There is a great deal of actual overlap in most of the structural characteristics of the calls among these species (fundamental being a possibly important exception), although the sum total of these characteristics yields a sound which may always be distinguished from that produced by either of the other species. Thus, there may be no single characteristic by which a frog distinguishes calls of its own species from others, but it may be the combination of all characteristics of a call which gives it its distinctive sound, which the female frogs discriminate. Three characteristics judged to be among the most important are employed in figure 4—fundamental, dominant, and paired or unpaired condition of secondary notes. It is seen that the symbols representing individuals of each species fall into rather closely-packed groups, quite well separated from the other species (one exception is a *phlebodes,* field no. 1158, whose frequency characteristics fall between those of *phlebodes* and *ebraccata;* morphologically this specimen cannot be distinguished from typical *phlebodes,* and the pattern of the call is that of *phlebodes*). This

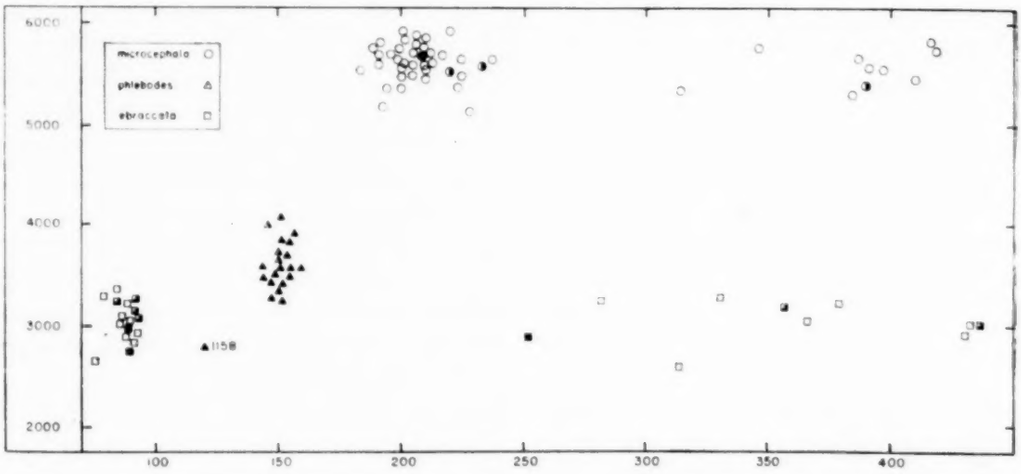

FIG. 4. Scatter diagram employing three important characteristics of the calls: fundamental frequency, indicated on horizontal axis; dominant frequency, indicated on vertical axis; and components of the secondary note, indicated as paired by clear symbols, unpaired by blackened symbols, or both types in one individual by half-blackened symbols.

degree of difference probably indicates an important ethological isolating mechanism.

The call difference may fail to isolate two species if the female does not discriminate between the two calls, and if the male does not discriminate in his choice of females. Littlejohn and Michaud (1958) demonstrated that it is unlikely that the female will fail to discriminate between the calls. They also found (personal communication) that the male shows no discrimination, attempting amplexus with any frog which contacts or hops near them. Thus it is probable that if a female enroute to a calling male should accidentally contact a male of another species, amplexus would occur. Additional ethological mechanisms could then be of importance in causing the male to release the foreign female. No experiments were performed with the species in the Canal Zone to test their amplectic compatibility, so nothing further can be added concerning ethological isolation.

Mechanical isolation. This is reproductive isolation resulting from the physical non-conformity of the pair of frogs involved. It is felt that probably the only true mechanical isolation existing in most frogs is due to a size difference so great as to make amplexus a physical impossibility. Because of the rather small differences in size, there is probably no effective mechanical isolation among the three Panamanian *Hyla*.

Post-Mating Mechanisms

Among the hundreds of frogs of these three species which I examined in the laboratory and observed in the field, no morphologically intermediate individuals were seen. Occasional specimens of *microcephala* showed a breaking-up of the lines of the dorsal pattern, tending toward the *phlebodes* pattern, but these were found to be good *microcephala* in all other respects, including call. Furthermore, of the amplexing pairs noted, no interspecific pairs were seen, although I have taken several amplexing pairs of all three species in situations where one or both of the other species were calling, sometimes only a few inches away. Thus, natural hybridization among these frogs is probably quite rare, if it ever occurs. In order to determine whether hybridization was biologically possible, artificial crosses were made in all possible combinations, using methods based on those of Rugh (1948). A few crosses were made in 1956, but at this time no suitable food had been discovered for the larvae, and numbers surviving at various stages were merely estimated. In 1958 more careful counts of survivors were made and a suitable food had been found so that the larvae were carried to more advanced stages. However, it was necessary to return to Texas prior to metamorphosis of the larvae, and all of them died in the laboratory at the University of Texas (including controls). Their death is now attributed to ammonia-poisoning (Hubbs, Littlejohn, and Littlejohn, 1960). In spite of the small number of crosses from which the data presented are derived (table 2), it is felt that the results give a good indication of the interfertility of the parental forms.

Gametic isolation. Fertilization occurred in all crosses except those between female *ebraccata* and males of the other two species. In the latter crosses, none of the eggs rotated or underwent any cleavage, so it is assumed that fertilization did not occur. Thus, in the event that all anti-mating and courtship isolation breaks down, gametic isolation appears to be 100% effective in preventing hybridization between female *ebraccata* and males of the other species. The relatively low percentage of eggs undergoing cleavage in the crosses ♀ *microcephala* × ♂ *phlebodes* might suggest some degree of lowered fertility, but it is felt that this is probably not true. Actually, in one of the two trials, 39 eggs of a total of 41 underwent cleavage, where as in the second trial only 9 of 73 cleaved. This low percentage in the second cross is thought

TABLE 2. *Results of crosses.* E = ebraccata, M = microcephala, P = phlebodes

Cross	Trials	No. eggs	Fertilized (cleavage)	No. hatched	Survival: normal+abnormal (total)			% hatched surviving 7 days	Rate of development
					72 hours	7 days	14 days		
♀E×♂E	2	70	63	63	63+0 (63)	63+0 (63)	63+0 (63)	100.0	control
♀E×♂M	2	93	0	—	—	—	—	—	—
♀E×♂P	2	97	0	—	—	—	—	—	—
♀M×♂M	2	206	179	66	66+0 (66)	57+2 (59)	54+1 (55)	89.4	control
♀M×♂E	2	98	82	59	26+20 (46)	1+3 (4)	0	6.8	retarded
♀M×♂P	2	114	48	42	35+2 (37)	29+1 (30)	29+0 (29)	71.4	accelerated
♀P×♂P	1	*e50	*e50	*e50	*e 50+0	*e 50+0	1+0 (1)**	100.0	control
♀P×♂E	1	*e50	*e45	*e45	*e 45+0	0+2 (2)	0**	*e4.0	retarded
♀P×♂M	1	*e50	*e50	*e50	*e 50+0	*e 45+0	*e 20+0	*e90.0	accelerated

* e = estimated; cross made in 1956, actual counts not made.
** = poor laboratory conditions and lack of proper food probably responsible for high mortality.

to be the result of faulty technique, namely over-crowding of the eggs.

Hybrid inviability. It is believed that in most crosses development proceeded far enough so that one might predict the degree of success of the cross, even though none of the larvae were carried to metamorphosis. In a number of preliminary feeding experiments with *microcephala* larvae, nearly all survived past the seventh day without feeding, so any deaths during the first seven days can probably be attributed to genetic causes. Thus it is felt that the figures for percentage of hatched larvae surviving seven days are reasonably reliable for all crosses, and may be used as an index of genetic viability.

Crosses between male *ebraccata* and females of the other species produced a large percentage of hybrids which were obviously deformed or abnormal, and many died in every stage of larval development. The rate of development (which was estimated by comparing the time it took for each cross to reach various embryonic stages, with that for the controls, as well as by comparing the general rate of gain in physical size) in the most "normal-appearing" of these hybrids was noticeably slower than that of either control. Less than 7 per cent of the hybrids that hatched were alive after seven days, and all were dead at the end of two weeks. It seems doubtful that any hybrid with *ebraccata* as one of the parents would survive to metamorphosis.

Reciprocal crosses between *microcephala* and *phlebodes* showed no more abnormal-appearing larvae than did the controls, and the percentage surviving past the seventh day are not much lower than those of the controls. The rate of development is faster than that of either control, especially in the earlier stages. The acceleration of development, plus the apparently higher survival rate of the hybrids after 14 days, suggest the possibility of heterosis. Thus hybrid inviability is probably not an isolating mechanism of importance between these two species.

Further hybrid breakdown was not tested because of the aforementioned death of the experimental stock.

Discussion and Summary

The breeding call seems to be the primary isolating mechanism operating to prevent interbreeding of the three species studied. All three species have calls which are demonstrated to be qualitatively and quantitatively different, and it is assumed that the females discriminate and respond only to the call of their own species.

In some areas differences in breeding habitat may support isolation, although there is complete intermixing in some areas. Two of the frogs (*microcephala* and *ebraccata*) differ in the site of oviposition, and this may lend additional support to habitat isolation. There may also be a difference in breeding peaks, although the actual breeding seasons overlap completely. A possible subtle difference in response to rainfall may help support isolation between two of the forms (*microcephala* and *phlebodes*).

If all these mechanisms fail, gametic isolation appears to be effective in interspecific crosses with female *ebraccata*, and crosses to male *ebraccata* result in early death of the few larvae that hatch. However, there appears to be no reduced fertility in crosses between the other two species, *microcephala* × *phlebodes*, nor any reduced hybrid viability, in fact there is a suggestion of heterosis. It is possible that these hybrids might die a genetic death before reaching maturity, but this is doubtful. There also remains the possibility of hybrid sterility and further hybrid breakdown, which were not investigated. However, in the absence of any records of natural hybridization, or even cross-clasping, it appears that reproductive isolation is effected by anti-mating and courtship mechanisms. Inasmuch as both species were commonly found calling and breeding within inches of each other, and because of the great overlap of most other factors which might support isolation, it is concluded that the only factor exhibiting a consistent significant difference, namely the breeding call, must be the primary isolating mechanism operating here. This is equally true of isolation between *ebraccata* and these two species. There is no reason to assume that this courtship mechanism will be any less effective merely because a potential post-mating mechanism exists. In fact, as pointed out by Fisher (1930), Sturtevant (1938), Blair (1955), and others, selection against the wastage of gametes will ultimately cause reinforcement of the potential anti-mating and courtship mechanisms in the area where these species come into contact.

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EVOLUTIONARY ASPECTS OF ABERRANT MEIOSIS IN SOME PENTATOMINAE (HETEROPTERA)[1]

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The normal males of most animals may now and then produce a few sperms that carry an abnormal number of chromosomes. In the great majority of cases these are the result of a mitotic accident that occurred somewhere in the course of development. However, there are some species in which aberrant sperms are not accidental but are produced regularly in a certain region of all testes.

Conditions such as these have been encountered in 21 species belonging to the insect Family of *Pentatomidae* (*Heteroptera*) and, more specifically, to the Subfamily *Pentatominae*. In most such species one entire compartment or lobe of every testis produces nothing but sperms with aberrant chromosome numbers. So bizarre are the meiotic deviations through which this is brought about that the testicular compartment in question has been called the "harlequin lobe."

All the evidence indicates that such sperms very rarely produce viable offspring and they therefore play at most an indirect role in the hereditary mechanism of the species. Nevertheless they are produced in huge numbers and it is an interesting question how so wasteful a feature could have become establsihed in so many species. The cytological features of the harlequin meiosis have been treated elsewhere (Schrader, 1960) and the present account is primarily concerned with the evolutionary aspects of the harlequin lobe.

[1] I am greatly indebted to Dr. H. Ruckes of the American Museum of Natural History for taxonomic aid as well as discussion of geographical distribution.

GONAD STRUCTURE

The gonad of the *Pentatomidae* consists of a number of compartments (ovarioles in the female, and lobes in the male) which, in the testis are held firmly together through their entire length by a stout, peritoneal covering. The normal number of lobes in the *Heteroptera* is 7 (Weber, 1931, 1933). Though deviations from this number—nearly always in a downward direction—are common, the variation is greater in some Tribes than in others. Thus the species of the *Halyini* vary from 4 to 7, and the *Pentatomini* from 3 to 7. But the known *Discocephalini* show no decrease from the typical 7 and, in the case of *Dinocoris rufitarsus,* have even added a lobe. By contrast the *Edessini* have a characteristically low number, with 5 as the top (in two species of *Edessa*) and 3 as the bottom (in a species of *Peromatus*).

It is more significant for the present inquiry that despite an apparent uniformity in the structure of the various lobes, conditions within them are by no means identical. For years it has been known that in many pentatomid species the dimensions of the spermatocyte cells vary widely in the different lobes. That is the case especially in the Tribes *Pentatomini, Halyini, Discocephalini,* and *Edessini* which, together, comprise about 4/5 of the approximately 3,000 species included in the Subfamily *Pentatominae*. Further, it holds whether a harlequin lobe is present or not; in *Arvelius,* which lacks such a lobe, the largest meiotic cells have nuclei that are eight times as voluminous as the smallest (Schrader and Leuchten-

TABLE 1. *Species with a harlequin lobe*

Order: *Heteroptera*; Family: *Pentatomidae*; Subfamily: *Pentatominae*	
Tribe—*Pentatomini*	
Brachystethus rubromaculatus	Costa Rica
Loxa flavicollis	Panama, Costa Rica
Loxa florida	Florida
Loxa picticornis	Panama, Costa Rica
Mayrinia variegata	Costa Rica
Pseudoevoplitus longicornis	Panama
Tribe—*Halyini*	
Alitocoris parvus	Costa Rica, Panama
Alitocoris schraderi	Costa Rica
Macropygium reticulare	Panama, Costa Rica
Melanodermus tartareus	Costa Rica
Moncus obscurus	Panama
Schraderia cinctus	Costa Rica
Schraderia hughesae	Costa Rica
Tribe—*Discocephalini*	
Ablaptus amazonus	Costa Rica
Agaclitus dromedarius	Costa Rica
Architas pudens	Panama
Dinocoris rufitarsus	Panama
Mecistorhinus panamensis	Panama
Mecistorhinus sepulcralis	Brazil
Mecistorhinus tripterus	Costa Rica
Neodine macraspis	Costa Rica

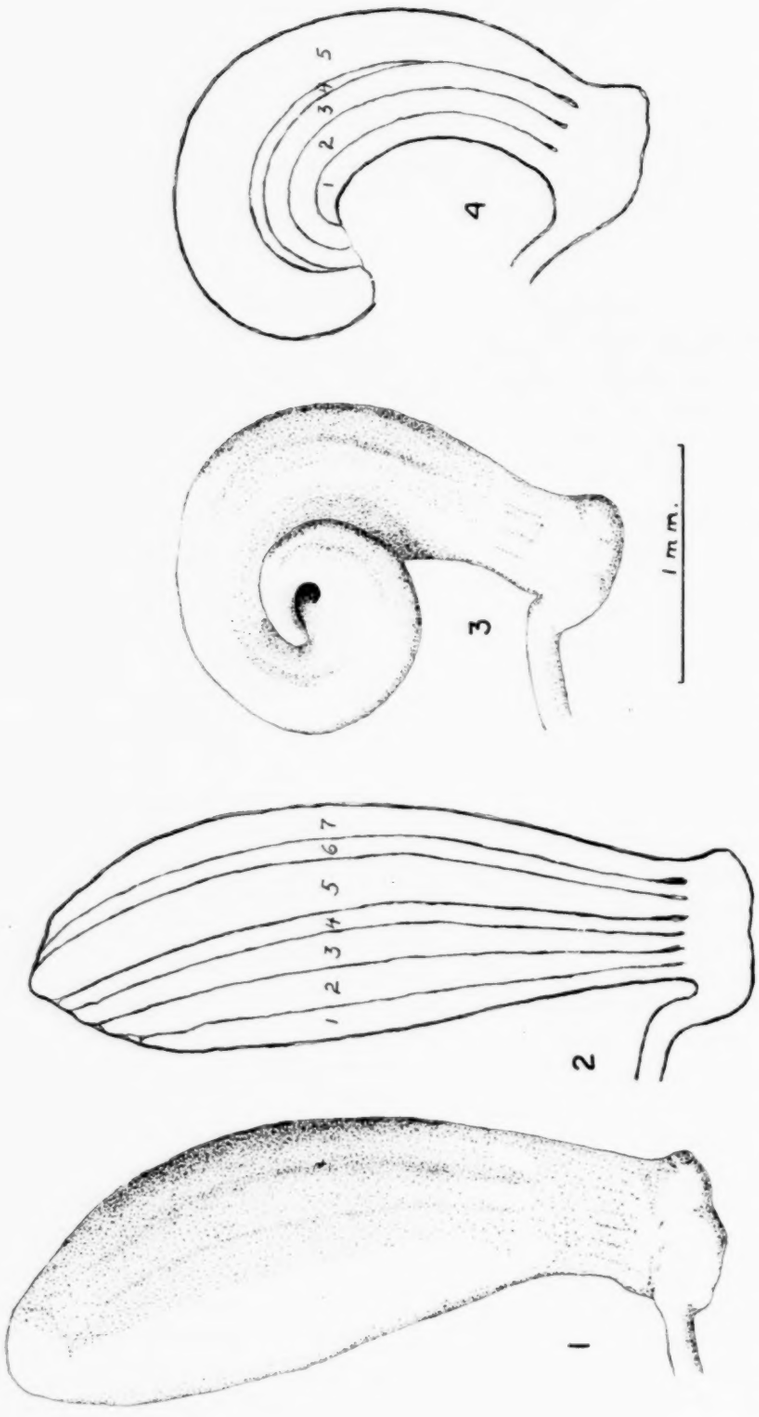
berger, 1950, 1951). These size differences are always linked with certain lobes and thus, in a testis with 7 lobes (which are arbitrarily numbered, starting with the lobe nearest the side at which the sperm duct enters) the spermatocytes of No. 1, 2, 3, and 7 have close to the common or normal dimensions, those of No. 4 and 6 are unusually large, and those of No. 5 are markedly smaller than normal. These size relations are constant not only within a given species but are also encountered in widely separated species, though this is not always immediately obvious in those cases where there has been an evolutionary loss of one or more lobes (fig. 2 and 4).

The size of spermatocytes bears no relation to the dimensions of the lobe. Indeed the "large cell" lobes are usually the least voluminous and the fifth or "small cell" lobe is much the largest (fig. 2 and 4). The latter, when it assumes the character of a harlequin lobe, becomes so disproportionately large in most *Halyini* and *Discocephalini* that it causes a twisting or coiling of the testis into the form of a "ram's horn" (fig. 3).

CYTOLOGY

Spermatogenesis

The cytological features of the harlequin lobe have been reported by a number of investigators (Schrader, 1945a, 1945b, 1946a, 1946b, 1960; Martin, 1953; Srivastava, 1957; Ansley, 1957, 1958) and a renewed description is hardly called for here. In essence, it may be said that the deviations from normal conditions that occur in the harlequin lobe affect especially the mitotic processes of the autosomes so that they are distributed irregularly in the maturation divisions. As a result, spermatids with abnormal numbers of chromosomes are produced—numbers which range from 1 (which is always a



FIGS. 1–4. 1. *Loxa flavicollis:* External view of testis. 2. *Loxa flavicollis:* Section of testis showing 7 lobes; No. 5 is the harlequin lobe. 3. *Alitocoris schraderi:* External view of testis showing coiled form assumed in some species with very large harlequin lobe. 4. *Alitocoris schraderi:* Section, showing 5 lobes; No. 5 is the harlequin lobe and No. 4 is degenerate. Tip of testis removed.

sex chromosome) to more than 100 (in *Loxa*).

The way in which this end result is brought about is, however, subject to some variation in different species. Such variation is most pronounced in the Tribe *Pentatomini* whereas there is considerable uniformity in the Tribes *Discocephalini* and *Halyini*. Except for a few cases of halyines where extreme harlequin conditions have not been reached, the meiosis in the harlequin lobe of these last two Tribes is identical in its main features. This similarity is of interest in view of the opinion recently expressed by Sailer (1950) and Ruckes (1958) that the taxonomic line of demarcation between the discocephalines and halyines can not always be drawn very sharply.

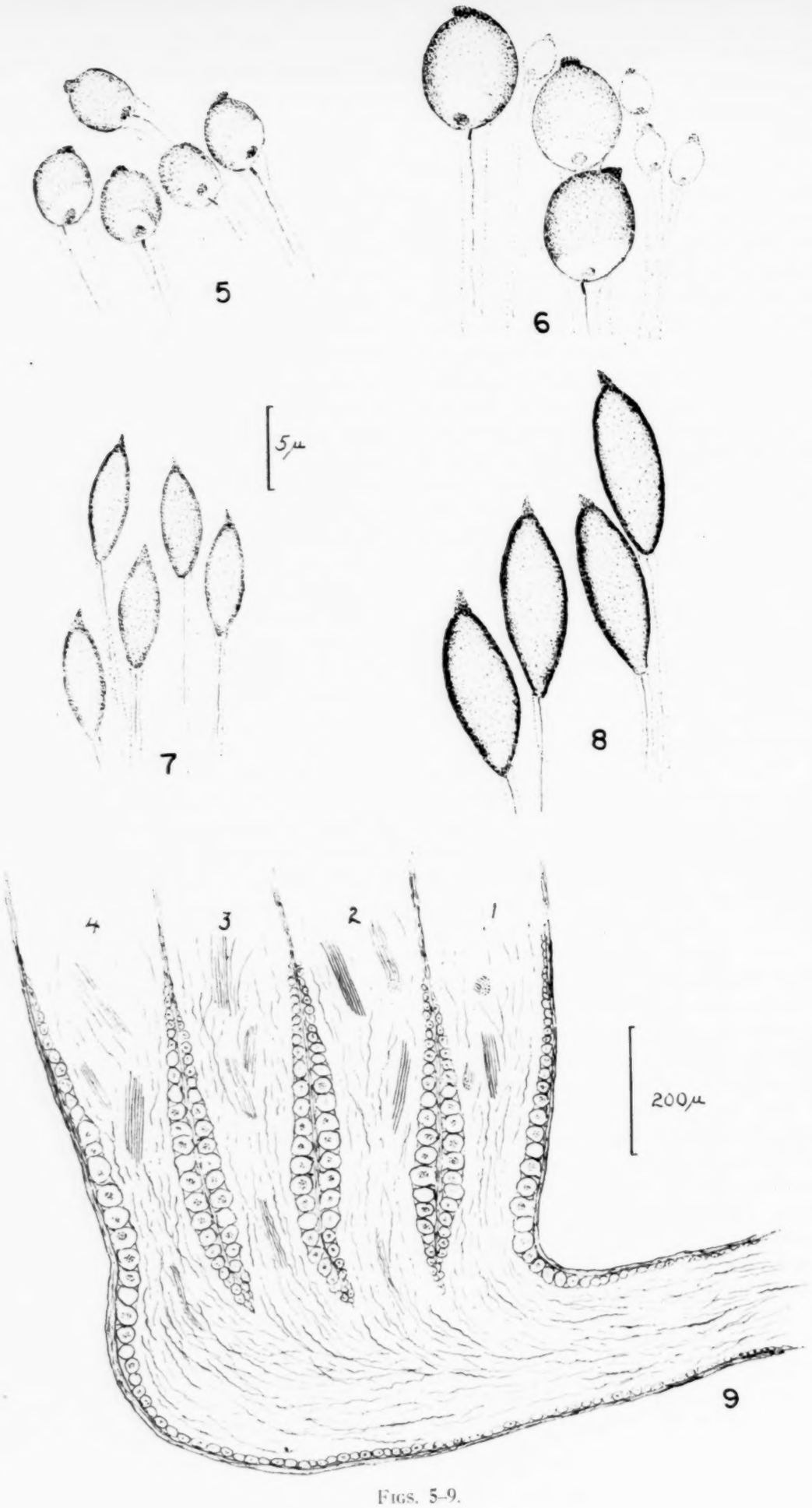
The whole situation is most strikingly portrayed in one of the *Pentatomini*, *Brachystethus rubromaculatus*, which will be used here as an example. In this species, three of the seven lobes have disappeared and the former fifth, now No. 4, has become the harlequin lobe. The meiotic prophase in the latter appears to be quite orthodox and the autosomes pair to form bivalents of normal appearance. But just prior to the first metaphase all the bivalents gather in a single clump (only very rarely are two clumps formed) and this clump remains quite intact through both meiotic divisions. The two sex chromosomes, X and Y, however behave perfectly normally; they divide equationally in the first and segregate to opposite poles in the second. As a result, only two main types of spermatids are produced: of every four spermatids derived from each primary spermatocyte, three carry nothing but a single sex chromosome, but the fourth carries a sex chromosome plus all the autosomes. Since in the orthodox meiosis the autosomal bivalents undergo two divisions, the large harlequin spermatids contain four times as much autosomal material as do normal spermatids. By contrast, the harlequin spermatids with nothing but a sex chromosome are of course greatly inferior to normal spermatids in chromosome content (Schrader, 1946a).

Despite these great deviations from the normal number of sperm chromosomes, the sperm formation that follows the meiosis in the harlequin lobe follows a perfectly regular course in its earlier stages. At this time the harlequin differs from the normal lobe mainly because there are such great size differences among the developing harlequin spermatids. In conformity to the chromosome contents the cysts in the harlequin lobe show spermatids that are exceptionally large among many tiny ones. In the normal cysts the size of the spermatids is very uniform (figs. 5 and 7) and there is no approach to the two extremes that characterize the harlequin cysts (fig. 6).

The further development of the harlequin spermatids can be followed readily until they have reached the stage shown in figure 6. At about this time the miniature spermatids seem to lose their staining capacity and then appear as pale shells. By the time that the spermatid nuclei begin to elongate it becomes more and more difficult to find these small ones whereas the giant spermatids continue development in a normal manner. When the latter have reached the stage shown in figure 8, the miniature spermatids can no longer be identified and, presumably, they disintegrate at about this time. In species where the harlequin meiosis results in spermatids with a more variable number of chromosomes (as for instance in *Agaclitus* and *Loxa*), those with only a few autosomes also tend to stain more and more faintly, and finally disappear (Schrader, 1960). In *Brachystethus* the harlequin lobe finally produces nothing but giant sperms.

The fertilized egg

The large harlequin sperms are extruded quite normally into the sperm duct (fig. 9) where they mingle with the sperms from the normal lobes. Due to the extreme length of all fully formed sperms it is almost impossible to dis-



FIGS. 5–9.

tinguish the harlequin from the normal sperms in the duct because, as Bowen found many years ago, it is a very difficult proceeding to trace any one sperm through its length among the twisted masses of sperms. But there can be no doubt that harlequin sperms pass down the sperm duct together with the sperms from normal lobes and that they have equal access to the eggs. However, as mentioned earlier, the extreme rarity of individuals with abnormal chromosome complements—none were encountered among over 100 specimen of the several species with harlequin lobes—is sufficient evidence for the fact that harlequin sperms seldom produce offspring.

But that does not signify that they play no further role after reaching the sperm duct. It must be remembered that polyspermy occurs in very many, probably most, insects and in some species the number of supernumerary sperms in the egg may be considerable. Huettner (1927) found that in *Drosophila melanogaster* "one can usually count a dozen or more sperms" within the egg, of which only one represents the male pronucleus that joins the egg pronucleus. All the available evidence indicates that the character of the chromosomal contents of the sperm affects its entry into the egg very little. That is not true of insects alone but also holds for other animals. Thus various investigators have found that though irradiated sperms of *Echinodermata* and *Amphibia* may bring about a highly abnormal development, their entry into the egg is affected very little (see for instance Hertwig, 1911). Only Ito and Leuchtenberger (1955) report that, in the case of the mollusc *Spisula,* "spermatozoa with a normal amount of DNA have a selective advantage over those spermatozoa which have a low amount" in entering the egg. But the latter are not shut out and Ito and Leuchtenberger have no data on sperms with excess DNA. All in all, it is very probable that the giant harlequin sperms will be found among the supernumerary sperms in the egg when fertilization takes place.

Evolutionary Aspects

As stated earlier, the harlequin lobe represents a waste that has become regularized and, moreover, reaches extraordinary proportions. In most species, the harlequin lobe is much the largest lobe of the testis and in certain forms such as *Alitocoris,* it comprises 2/5 or more of the testis volume. This means that a very considerable part of the reproductive organ no longer functions in its original capacity. It is a question of some interest how such a situation could have arisen.

Role of harlequin sperms

The existence of a harlequin lobe in so many species belonging to three different Tribes of the *Pentatominae* suggests that its origin involves some basic conditions that originally were present in the Subfamily as a whole. These conditions are still indicated by the constant size differences of the spermatocytes that are always associated with certain lobes. The various sizes are due to the different amounts of proteins, polysaccharides and RNA that they contain and this clearly shows that the synthetic activity in the different lobes is not the same (Schrader and Leuchtenberger, 1950, 1951).

It is also clear that it is always a certain one of the lobes that assumes the harlequin condition. That is the "small cell"

Figs. 5–9. 5. *Brachystethus:* Spermatids in normal lobe, all of same size. 6. Spermatids in harlequin lobe of two sizes; small ones carry only a sex chromosome, large ones carry sex chromosome plus all the autosomes. 7. Spermatids in normal lobe in early phase of elongation. 8. Spermatids in harlequin lobe in early phase of elongation; those of small size no longer recognizable. 9. *Brachystethus:* Lower end of testis, showing the 4 lobes opening into sperm duct. Sperms from No. 4, the harlequin lobe, enter the duct just like those from the 3 normal lobes.

lobe or No. 5 as it is represented in species that retain the normal number of seven lobes. In short, the harlequin lobe evolves from a lobe that differs from its adjoining neighbors in a very specific aspect of its physiological processes.

Why it has persisted is another question. Since it involves a waste that goes beyond the ordinary, its elimination by natural selection might well be expected. As a possible explanation of its widespread existence it has been suggested (Schrader, 1946a, 1960) that the large harlequin sperms, though not functioning in fertilization proper, may nevertheless be of benefit to the species. This possibility is strikingly presented in *Brachystethus* where the evidence indicates that of the harlequin sperms only those carrying a much greater quantity of nucleoproteins than the normal sperms ever reach the sperm duct.

Their further role is contingent on the polyspermy that occurs in most insect species. Polyspermy, in itself, probably became established because the breakdown of supernumerary sperms in the egg releases substances such as nucleoproteins that undoubtedly are utilized by the embryo. If the addition of these valuable materials confers an advantage on species with polyspermic eggs, such an advantage would be considerably enhanced if some of the extra sperms are derived from a harlequin lobe. In the case of *Brachystethus,* a harlequin sperm would contribute almost four times as much nucleoprotein as does a normal sperm and it is this extra contribution that may have played an important role in the persistence of the harlequin lobe.

Geographical distribution

All except one of the 21 species in which a harlequin lobe has been found, are tropical (*Loxa florida* is subtropical). What is the import of such a distribution? The situation is not quite the same in the three Tribes involved and they will be taken up separately.

Pentatomini. A harlequin lobe has been found in 6 species of this Tribe and all 6 are Neotropical. This fact is impressive when it is considered that the cytology of more than 100 species of the *Pentatomini* has been studied and that none from the Palaearctic and Nearctic regions (which represent the great majority) has been reported to have a harlequin lobe. It should be noted however that our information on Oriental pentatomids is limited to some Indian species, and that no cytological work at all has been done on the Pentatomidae of the Ethiopian and Australian regions.

It is conceivable that harlequin lobes were overlooked by some earlier investigators but this is not likely in the case of E. B. Wilson who reported many instances of special chromosome behavior, or Bowen who was the first to recognize the harlequin lobe of *Loxa* (1922a). Their investigations together with my own constitute a considerable section of the evidence that is trustworthy in this respect.

This means that, so far as is known, a harlequin lobe is lacking in all *Pentatominae* that live in the temperate zone. It is present only in species that have a tropical habitat, but by no means all tropical species possess it. Thus, of the Neotropical *Pentatomini* known to me, it is absent in 3 species of *Arocera,* 3 of *Acrosternum,* 5 of *Banasa,* and 1 each of *Arvelius, Agroecus, Pellaea, Thyanta,* and *Tibilis.* In short, if all species with a harlequin lobe are tropical, the converse does not hold and the correlation is not absolute. The need for more data, especially for *Pentatomini* from the Ethiopian and Australian regions, is patent.

Discocephalini. By contrast with the preceding Tribe, this has considerable uniformity. Its species are confined to America and almost entirely to its tropical region. Of the 9 species investigated, 8 have a harlequin lobe. The ninth, *Platycarenus humilis* (mistakenly identified as *P. notatus* in my 1946b paper), shows some exceptional cytological fea-

tures but these are not directly related to a harlequin development.

It is however pertinent to note that *Platycarenus* is the only discocephaline genus that is represented by a species recorded as definitely outside of the tropics. The exception is *P. tenebricornis* (Ruckes, 1957) which occurs in southeastern Arizona in the United States. In short, the only discocephaline genus that includes a species lacking a harlequin lobe is not as strictly confined to the tropics as are other genera of the Tribe which do have such a lobe.

Halyini. All 7 of the tropical species that have been investigated have a harlequin lobe. In *Alitocoris, Macropygium,* and *Schraderia* its deviations from the normal are not as great as in most other *Pentatominae,* but in *Melanodermus* and *Moncus* it reaches the extreme development that is also seen in the *Discocephalini.* The intermediate character in the first named 3 species suggests that an experimental attack may give some clue to the factors that cause the fifth or "small cell" lobe to assume the harlequin condition.

But though every halyine species from the Neotropics has been found to have a harlequin lobe, such is not the case with the only Nearctic *Halyini* whose cytology we know. These are *Brochymena arborea* and *B. quadripustulata* which are definitely non-tropical and indeed have a range extending into Canada. Both of these northern species have a normal testis without harlequin development. The existence of several purely tropical species of *Brochymena* would seem to represent a natural experiment and it would be a matter of high interest to determine whether such species as *B. cuspidata* and *B. tenebrosa* which are confined to the Neotropics (Ruckes, 1947), have any indications of a harlequin development.

But the *Halyini* are not found only in the Americas, being also well represented in the Oriental, Ethiopian, and Australian regions. Unfortunately nothing is known of the cytology of any halyine from the two last named regions. From India a number of reports on Oriental *Halyini* are available (Manna, 1951; Rao, 1954; Sharma and Parshad, 1956; Parshad, 1957). The species investigated are *Dalpada clavata* (*concinna*), *D. confusa, D. versicolor, Halys sulcata* (*dentatus*), and *Halys sp.* However in none of these papers except the last is the possibility of a harlequin lobe mentioned and it is conceivable that, in an endeavor to establish the normal conditions, it may have been overlooked. Only Parshad (1957) states specifically that in *D. clavata* and *D. confusa* no abnormality suggesting a harlequin lobe exists. Rao mentions a "pseudo bivalent" in *D. versicolor* but this is no indication of any aberrant chromosomal behavior for it is obvious from his account that he applies this term to the ordinary "touch and go" pairing of the X and Y that is almost universal in the *Pentatomidae.* All in all, the evidence on Indian *Halyini* is not entirely adequate. Moreover, of the *Halyini* investigated, *D. versicolor* appears to be the only truly tropical species.

Here again, therefore, our data on tropical species are almost entirely derived from examples of the American tropics. Information on halyines from other zoogeographic regions is badly needed.

Ecology

Our knowledge of ecological factors that might throw light on the evolution of the harlequin lobe is extremely limited. Very little work has been done on the life history of tropical *Pentatominae* and that little is mainly confined to my own observations on the *Halyini.*

All 7 of the *Halyini* mentioned in the present account are usually found in dark locations. The specimen of *Alitocoris* and *Schraderia* were collected from under leaves or bark lying on the ground. Frequently they had buried themselves in the loose soil under such objects. *Macropygium* was commonly found in the earth

among the shallow roots of "chayote" plants (*Sechium edule*), a cucurbit widely cultivated in Costa Rica. It would there seem likely that such species are root feeders to some extent. In seeming conformity to this mode of life these 7 halyinids constitute an aggregation of sombre coloring, their dark grays and browns being only occasionally relieved by subdued yellow edgings and spots. Frequently, one or two mites are attached to various parts of the body which, as in case of certain *Coleoptera,* may also be correlated with life on or in the soil.

But though these 7 halyinids thus show some uniformity in a mode of life that is not shared by most other *Pentatominae,* its bearing on a harlequin role is not obvious. Certainly, some species in the other Tribes show no correlation between a harlequin development and such ecological features as have been mentioned. For instance, *Neodine, Loxa,* and *Brachystethus rubromaculatus,* can not be considered as having a subdued coloring, and the latter two are commonly encountered among foliage high above the ground.

Conclusion

There would seem to be little doubt about some of the steps that led to the establishment of the harlequin lobe. They can be summarized as follows:

1. The fundamental organization of the testis in the Subfamily *Pentatominae* involves constant differences in the metabolic chemistry of the various lobes that comprise the testis. These are expressed by the different size of cells that is attained in each lobe.

2. Lobe No. 5, as it is present in the typical pentatomid testis, is characterized by abnormally small cells.

3. The harlequin lobe represents an alteration of the physiological conditions in the fifth lobe. One cannot say whether the testis as a whole has been subject to change in its chemistry and that the fifth lobe is merely the most sensitive of the lobes, or whether the change was restricted to the fifth lobe. Either is possible.

In any case an origin from the fifth lobe is rather well established and it was a change in its chemical conditions that brought about the meiotic irregularities of the harlequin lobe. Since the latter constitutes a considerable section of the testis—often 2/5 or more—the question arises as to its persistence in so many species. The evidence indicates that usually only harlequin sperms that contain more chromosomal material than normal sperms, reach the sperm duct and have access to the egg. In the polyspermy that characterizes most insects, these large harlequin sperms probably constitute a percentage of the extra sperms in the egg. When supernumerary sperms are broken down their contents, mostly in the form of nucleoproteins, are undoubtedly utilized by the developing embryo. Since the harlequin sperms contribute larger quantities of such readily available substances to the embryo, it is conceivable that this additional contribution that they make to the nutrition of the embryo may constitute one factor in the persistence of the harlequin lobe.

The fact that all 21 species, in which a harlequin lobe has been found so far, are tropical or subtropical in habitat is given added significance by the absence of such a lobe in the many *Pentatominae* from the temperate zone. The agency of a constantly high temperature must therefore be seriously considered in its evolution but it is obvious that there are other factors in a tropical ecology that may have played a role. We are at present sadly lacking in information about the biology of tropical *Pentatominae.*

Whether the change of the fifth to a harlequin lobe occurred before the *Pentatominae* split up into Tribes and thus represents a remnant of ancestral conditions, or whether it has arisen independently in the *Pentatomini, Discocephalini,* and *Halyini,* cannot be decided at present. Though the first named alternative

would seem to be the most likely, the possibility of an independent origin in the three Tribes should not be dismissed entirely. The general harlequin conditions show some similarities wherever they occur. Though this may also bespeak an origin in a common ancestor, it is at least conceivable that the change in the fifth lobe does not involve a complex series of steps and therefore may have occurred repeatedly. A final decision between these two alternatives cannot be made.

It should be emphasized that these cogitations concern only tropical American species. Information about *Pentatominae* from other tropical zoogeographical regions is sadly lacking and urgently needed.

Summary

1. The harlequin lobe, which constitutes a considerable portion of the testis in some *Pentatominae*, usually produces nothing but aberrant sperms.

2. The harlequin lobe represents a modification of the fifth lobe in the normal testis, which itself differs from other lobes in its synthetic processes.

3. The persistence of the harlequin lobe may be contingent on the polyspermy that characterizes most insects. Extra sperms in the egg are broken down and no doubt are added to the nutritive materials available to the embryo. The harlequin sperms that reach full development usually carry more such materials than normal sperms and this may have been a factor in the establishment of the lobe.

4. The 21 species of *Pentatominae* which have been found to possess such a lobe, are all tropical or subtropical in habitat. No harlequin lobe has ever been reported in species from the temperate zone and the significance of that fact is discussed.

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EVOLUTION AND CYBERNETICS

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All living systems, individual organisms, populations, entire species, as well as biocoenoses, possess a certain ability for auto-regulation of their structure, i.e., they are homeostatic. They alter their structure with a change of environment, and restore it upon the restoration of previous interrelations. Living systems also change their structure with historical transformations of the interrelations between the organisms and their environment, and these changes are of an "adaptive," i.e., clearly regulatory character.

These facts justify an attempt to consider from the cybernetic viewpoint (Wiener, 1948) not only the individual development of organisms but also the mechanism of evolution. In the present analysis we shall hold to Darwinian conceptions as follows.

The primary object of evolutionary transformation is only the population. The directive information derives from the environment (biogeocoenose) by means of reproduction of tested individuals. This information undergoes transformation in the course of development of the phenotype. The controlling, feedback information on the actual condition of the population is transmitted to the biogeocoenose through the specific forms of the activity of its individuals. Control and transformation of the feedback information occur in the biogeocoenose which includes the given population. The specificity of the transformation is determined by the population structure and is attained by means of natural selection. Direct information on the results of transformation is transmitted through the hereditary mechanism of each zygote of the new generation of a given population. This is the generalized scheme of the elementary cycle of evolutionary transformations (Schmalhausen, 1958 a, b, c; 1959).

Analysis of the evolutionary process from a new standpoint allows us to divide the elementary transformation cycle into separate steps and enables us to distinguish the main factors determining the operation of each of them. Furthermore, it becomes possible to apply information theory methods to the quantitative evaluation of biological phenomena.

Mechanism of the Transmission of Hereditary Information

The mechanism of hereditary transmission was well known long before information theory was elaborated. However, the organization of the complicated inheritance mechanism became fully comprehensible only with the introduction of new concepts (Shannon and Weaver, 1949). Transmission of hereditary information occurs only in the process of cell division. All permanent cellular substances, both nuclear and plasmatic ones, have a role to play in this. However, the existence of a strikingly exact mechanism of copying, separation and equal distribution of chromosomes in mitotic division clearly shows the key importance of this particular mechanism. The chromosome structure, which led Schrödinger (1945) to compare it with a code, was particularly thoroughly studied in Drosophila, genetically, and then corroborated in a remarkable way cytologically. However, it was the study of the chemical structure of DNA (Watson and Crick, 1953) as the main substance of the chromosomes, that was of decisive importance in this respect. Although this topic needs much further elucidation, the

general scheme is sufficiently clear to enable us to evaluate fully its biological significance.

A DNA molecule, according to this concept, consists of a paired chain of nucleotides held by very weak hydrogen bonds. This makes possible the longitudinal split of the whole molecule with subsequent restoration of missing partners in each of the chains. Such a splitting and restoration is the basis of the biological process of self-reproduction, and, at the same time, the basis of the mechanism of the transmission of hereditary information. Nucleotides possess a similar composition of sugar (ribose), phosphate and one of four different purine (adenine or guanine) or pyrimidine (thymine or cytosine) bases, with adenine paired only to thymine, and guanine only to cytosine. These pairs can be repeated along the whole molecule in two different positions, so that each link in the chain can be one of four kinds (AT, GC, TA, CG). This permits the alternation of chemical structures resembling that of the signs in Morse code which is transmitted also by means of four different signals (dot, dash, space, double space). The possibility of having one of four different signs at each of several thousand links (along the whole DNA chain) allows the formation of a practically indefinite number of individual combinations. As a matter of fact, however, both in the telegraphy, and in the hereditary code, only a small number of all possible combinations is utilized. In both cases the information is transmitted by means of signals coupled into definite blocks. In telegraphic messages it is the letters and figures transmitted by means of a consecutive set of impulses. In language transmission vocal or written symbols are united to still larger blocks, the words. And finally, words are coupled in a certain manner into sentences which do not allow any random rearrangement without disturbing the sense of the message. This linkage of the primary signs contributes to a considerable increase in the comprehensibility and reliability of the entire transmission.

The pairs of bases (AT, GC, TA, CG) are the primary signs in the hereditary information. These units of chemical information make up the nucleotides which are linked into blocks called genes, which serve as biological information units (comparable with letters or even with words of language transmission). The complexity of gene structure has been proved in some cases. But usually genes are transmitted as indivisible units. They may be recombined (as the words in language transmission), but this disturbs the hereditary transmission and in many cases the new combination turns out to be inviable. Since exact transmission of hereditary information is of key importance for self-reproduction and evolution of organisms, it is protected by further intra-systemic connections. That is to say, genes are blocks of information but they are combined into larger blocks (supergenes, polygenic blocks) and, finally, into chromosomes. These undergo reproduction and are transmitted from one cell to another and from one generation to another usually without disturbing the sequence of information elements composing them.

Finally, the whole set of chromosomes typical of the given species of organisms is transmitted as an entity by means of the complex mechanism of mitotic division. From the viewpoint of information theory, the set of chromosomes is a complex message consisting of many symbols united into long blocks.

Any transmission of information may be interfered with. As in other cases, interference in the transmission of hereditary information is caused by disturbing influences of environmental factors. Hereditary changes, i.e., mutations, are a result of such interference or noise. Sources of origin of hereditary variability are well known. Ionizing radiation, some chemical agents and thermal shocks, are the common means of experimental production of hereditary variation. But, muta-

tions appear in nature without mediation by such drastic agents. It is reasonable to assume that it is the influence of some external factors that is responsible for mutability in such cases. At any rate, such influences are of low specificity and are restricted to elementary changes at some point on the chromosome, i.e., they involve only one or a few chemical information units within a single gene. This indicates that the action of an external factor is manifested only on the molecular scale, so that its localization is at random.

Although hereditary variations are utilized in evolution, a certain stability of organization and the possibility of its exact reproduction in the progeny are of great importance. Thus, the viability of a population depends first of all on the stability of organization since most mutations are damaging to individuals. Furthermore, exactness of organization allows it to be tested in many ways and repeatedly during different seasons, under different local conditions and in subsequent generations. Such testing is the basis of natural selection in the "struggle for existence," and therefore of the progressive evolution of organisms.

Thus, transmission reliability of the hereditary information is of great significance not only for the life of populations, but also for the possibility of their evolution. Hence, means of protection against possible disturbances in transmission of information have been elaborated. Firstly, the entire transmission system of hereditary information, i.e., the mechanism of chromosome self-reproduction and mitotic division, was improved in the process of evolution. Then, the means of protection against the disturbing influence of accidental external agents were developed. It is interesting to note that in this process the same means were used which communication engineering applies to noise abatement. Among them establishment of systemic connections in the form of information blocks stands foremost. Long blocks of binary signs are considered to provide the most economic coding system. In the long DNA molecule only two pairs of bases are alternating. Very long blocks are also found in chromosomes. Each gene can have only one of the two possible meanings in one of the homologous chromosomes of a diploid individual. In daily practice, in broadcasting in particular, a common means of increasing the reliability of information is the repetition of the whole message or of its most important part. This method is also utilized in the transmission of hereditary information. Similar genes (polygenes) or apparently identical chromosome segments (repeats) are often found in a chromosome. Alteration of a single gene in such a system often does not lead to substantial changes in the hereditary information or the structure of the organism as a whole. The diploidy of organisms, i.e., the repetition of the entire hereditary code, is a nearly general means of protection against the disturbing effect of noise. It is natural that "noise-resistance" of polyploids, often found in plants existing under severe conditions of the Arctic or under mountain or sharply continental climates, is even higher. Finally, simple isolation from the disturbing effects of external factors is also of great importance, just as in communication engineering. The hereditary DNA code is well isolated by the protein cover of chromosomes, the nuclear membrane, and by the regulating mechanisms of the cell and of the organism as a whole. Because of this isolation, external factors in their specific form never reach the hereditary code (only repeatedly reflected influences are possible, and they are manifested in the form of discrete mutational changes).

Amount of Hereditary Information

The amount of information is measured by the number of possible variants of a message, i.e., by the amount of possible combinations from a given number of symbols (Goldman, 1953). Theoretically, the possible combination number

(k) increases exponentially depending on the total number (n) of the signs used. In the logarithmic form of the equation, $k = L^n$, the logarithm of the number of possible combinations is directly proportional to the number of the signs used. Hence it is the logarithm of the number of the information units or symbols that is the most convenient measure of the amount of information, $\log k = n \log L$. As this value depends on the number of the signs used, i.e., on the length of the message, it is convenient to convert it to the basis of average information per sign: $H = \log L$ (where L is the number of symbols, i.e. of different kinds of signs used if equally frequent, or more generally the reciprocal of the frequency of a particular symbol). This holds for the particular case of random combination, and, therefore, for equally probable combinations. But actually, combination is not at random either in language transmission or in that of hereditary information. Although linkage of gene systems in the hereditary code does not rule out the possibility of recombinations, it does provide a hindrance and to a different extent for different signs (genes). In such cases the amount of information conveyed by n signs (or a single gamete for example) is measured by the sum-total of negative logarithms of the probability of each symbol, $I = -\Sigma \log p_i$. Under equal probabilities of different symbols (for example, independence at different loci in a gamete) this is reduced to the expression n log L as in the preceding formula. To obtain the amount of average information per sign, each logarithm in this summation is multiplied by its probability: $H = -\Sigma p_i \cdot \log p_i$.

It should be noted once more that when measuring the amount of information, only the actually realized variants (mutant genes) are considered. This means that only actually observed hereditary variations are taken into consideration. This is important, since the full complexity of the hereditary code cannot really be ascertained. We never know the total number of hereditary units or genes. Therefore, the amount of hereditary information of a gamete, *considered in relation to all possible gametes produced by a given individual,* is evaluated in a simple way. Only heterozygous genes segregate; hence each of the two germ-cell variants is equally probable. The amount of information at one locus in a gamete from an individual heterozygous at this locus is $\log_2 2 = 1$, and the total information simply the number of heterozygous genes in the parent. For our purposes, however, of much greater importance is measuring hereditary information in a total population, and when the genetic composition of the population is known, this presents no difficulties. Where the alleles at different loci are not independent (as might be the case for example in cases of linkage and selection) the same expression, $I = -\Sigma \log p_i$, suffices, but p_i now must be interpreted as the frequency of a given gene in a population, and the summation is over all genes.

The average information per gene, $H = -\Sigma p_i \log p_i$ is, however, a more convenient measure, its value depending mainly on the distribution of not infrequent genes and of common genes, that are readily identified. The amount of the average hereditary information per gene is an important measure characterizing the genetic structure of a population (and of the entropy of the variant distribution).

Transformation of Hereditary Information in Individual Development

In terms of information theory individual development means a transformation of the direct hereditary information transmitted through cell divisions at the level of the DNA molecular organization, into the feedback, phenotypical information transmitted at the level of organization of entire individuals. The hereditary information code performs here a dual function. On the one hand, it is reproduced and transmitted to daughter cells;

on the other, it plays the most responsible role in the transformation itself. The hereditary code determines, partly directly and partly indirectly, the synthesis of nucleic acids, of protein substances and of specialized enzymes of the cell. The latter, in turn, controls the course of metabolic processes, and the synthesis of various metaplasms, depending on the position of the cell and its connection with the surrounding "medium." In the long run all these processes are determined by the entire hereditary code. However, individual local code variants (mutant genes) play the role of "signals" which bring about deviations from the normal course of these processes.

Information theory has its methods of quantitative evaluation of variants, enabling us to compare the amounts of hereditary and phenotypic information in populations of a given species of organisms. Both the theory of information and the theory of natural selection evaluate only the variants, and not the whole "norm," and, furthermore, not the variants of individual characters, but only the variants of the organization as the whole. This makes the comparison of phenotypes with genotypes easier, since such complicating phenomena of phenogenesis, pleiotropy and polygenic determination of characters are thus excluded. Each change in a gene leads to some change in the mechanism of individual transformation and, possibly, its outcome. Any mutation can manifest itself as a change in the whole phenotype. This possibility finds its most complete expression in haploid organisms with a mosaic mode of development.

In the limit, phenotypical diversity of forms is determined by hereditary diversity, while the amount of phenotypical information equals the amount of hereditary information. In reality, however, the processes of individual development depend both on external factors and on the internal organization of the developing system itself. Common environmental factors enter, in one way or another, the normal mechanism of individual development and do not bring about appreciable deviations (or produce definite modifications in the whole group of individuals entering the sphere of influence of the given factors). Usually they do not increase the total amount of phenotypical information. On the other hand, the mechanism of individual development has to a degree a regulating character. The usual dominance of the norm (wild type) is one of the expressions of this fact. Most regulating mechanisms lead to the decrease in the diversity of form, and hence, to a decrease of the amount of the average phenotypical information per individual. It may be assumed that this amount is, in general, lower than that of hereditary information. This is of great importance for the process of evolution, since natural selection acts on phenotypes, so that even under a complete phenotypical uniformity of a population (due to severe selection) a considerable potential reserve of genetic variability still exists, especially in diploid and polyploid organisms.

The total system of phenotypic traits is the final result of the successive transformation of hereditary transformation in the developing soma. This system is the bearer of feedback information from the individuals of a given population to the biogeocoenosis which it enters. The feedback information is in the form of symbols, which are the phenotypic traits or characters of an individual, just as the direct information is in the form of symbols, genes, in the hereditary code. There is nothing in common between the two different sets of symbols, the characters and the genes, though their relationship as the result of the transformation, i.e., of the transition from one coding system to another, is beyond any doubt. This transition is so complicated that we cannot say that a certain gene brings about the development of a certain character. It is the code as a whole which determines the development of the phenotype as well as of each of its characters. The altera-

tion of one information element (gene) leads to a change in both the whole transformation process and the whole phenotype. Regulating mechanisms of a developing system may, however, make a mutation have either no visible expression, or manifest itself in the disturbance of only some less "protected" processes, which will produce the impression of change in single traits minimizing the change in the whole organism.

It was already noted that the hereditary code is not only the carrier of information but the active controller of cell metabolism, i.e., of the basic processes of life. Now it should be pointed out that the phenotype of an individual is certainly not merely a passive carrier of feedback information. On the contrary, it is the sole independent representative of life and an active participant in the attack by the population on the vital resources of the environment. If the hereditary code controls cell metabolism, the phenotype controls the metabolism of the whole individual.

Phenotypical Information and its Evaluation

Transmission of feedback information on the population is carried out through the individuals composing it. Each individual, though representing a complex message (which can be deciphered by studies on developmental genetics) is, at the same time, an indivisible biological unit, i.e., an entire block of information. The specific form of the activity of each individual in a certain environment (biogeocoenose) is the means of transmission of feedback information.

The basic manifestation of this activity lies in the procurement of the means for existence related to reproduction and protection of both the individual's own life and that of its progeny. Activity of individuals is specific, since members of a given species consume specific inorganic and organic substances and enter certain interrelations between one another and with other components of the biocoenose. At the same time, this activity is individualized and expresses peculiarities, tastes and habits of a given individual. The individuals of any species affect the biogeocoenose by their whole activity and thus "inform" it regarding the state of the given population.

External factors disturbing transmission of information are called noise. In the case of phenotypical information from a population to the biogeocoenose, the noise is represented by all inorganic and biotic factors destroying the carriers of this information, reducing or inhibiting their activity and reproduction, and thereby reducing and distorting feedback information. Various means of noise abatement have been developed in the process of evolution. As in other cases, "noise-resistance" is increased by the establishment of systemic connections between the information units, by means of the repetition of the message, as well as by means of direct protection and isolation against disturbing factors. The passive and active protection of the life of individuals by means of "isolation," i.e., development of hard integuments, thorns, spines and prickles, of venoms, as well as by means of adaptations and instincts permitting utilization of shelters from injurious climatic factors, attacks of predators and parasites, are very common means to increase noise-resistance.

Individuals, as a rule, are independent information units. However, temporary or permanent connections can be established between them (herds, packs, flocks, colonies), and as in the case of other relations between information elements, they lead to an increase in noise-resistance. Pairing of individuals of different sexes also results in more reliable information transmission.

Individuals of a given population (and of a species as a whole) are carriers of similar information. This ensures considerable repetition and, therefore, reliability of information on most significant characters. At the same time there is no repetition of characters of secondary im-

portance, allowing a range of differences between individuals in them. Individual diversity makes the composition of the population very labile so that it readily changes in the process of evolution.

The extent of diversity of individuals can be evaluated by methods of information theory. Evaluation of variants, as "events" which are far from being equally probable, can be performed only when their frequencies in a population are taken into account. Since frequency (probability) is always a fraction and its logarithm has a negative value, the amount of information is expressed only by positive values which increase with the decrease in frequency. Since very rare variants might be neglected (at any rate, their frequency cannot be exactly determined), it is the value of the average information per individual which has to be used for the calculation of the amount of information in natural populations. This value is obtained by multiplying the amount of information brought in by each variant by the frequency of this variant: $H = -\Sigma\, p_i \log p_i$.

The value of the average information contributed by each variant changes regularly with its spread in a population from zero, when it is completely absent, to zero, when it is the only form found in the population. The maximum value is reached not at the average frequency of $p = 0.5$, but at a much lower level of $p = 0.368$. This is connected with the fact that in information theory an evaluation of each variant is made through the negative logarithm of its frequency or probability of occurrence. This evaluation is a logarithmically decreasing quantity: the variant becoming more widespread loses its "novelty" on the background of the whole population. The deficiency of concentration at $p = 0.368$ (in comparison with 0.5) is still compensated by the qualitative advantage of the variant (its novelty). At higher frequencies this qualitative advantage is lost, the variant becomes increasingly more common, and its free choice becomes ever more compulsory. All this is merely a consequence of the accepted methods of information evaluation. It is important to note that this evaluation has also a biological significance which will be referred to later when discussing natural selection.

Control and Transformation in the Biogeocoenose

A population affects the biogeocoenose the composition of which it enters, through the activity of its individuals, constituting the feedback information on the actual state of the population. Activity of individuals is controlled by factors of the biogeocoenose which are extrinsic with respect to the population. These factors may be divided into two categories of essentially different significance: means for existence and "noises." Life-resources and their availability are of principal importance not only for the existence of a population but also for its evolution. They aid in the supply of energy for the transmission system and the transformation of information. The noises, i.e., accidental external factors, producing more or less adverse effects on the life and reproduction of a population, are of subordinate significance.

Procurement of the means for existence by individuals is controlled and limited by their presence in the biogeocoenose, in particular, by food resources and their availability. Maximum utilization with minimal energy consumption is the source of strength and resistance of the organism. The more active individuals develop normally and leave progeny. The less active and, therefore, less resistant individuals, perish because of predators, diseases and climatic adversities. The outcome, when food resources are limited, is determined mainly by the particular properties of individuals. Their evaluation or testing is a relative matter, which may be called competition. Competition goes on between the individuals within the population, and its results are determined by a complex interaction between external and internal factors.

The object of control is the phenotype of an individual, while the means of control are the environmental factors which each individual encounters in its activity. The controlling mechanism of the biogeocoenose represents precisely Darwin's "the struggle for existence" which is associated with natural selection.

Since the phenotypes reflect hereditary properties of individuals (under given conditions of development), the results of this control expressed in the natural selection of "fittest" individuals turns out to be rather complicated. The controlling mechanism of the biogeocoenose manifests its direct action only on phenotypes, but, through it, is also the means of control of: 1) hereditary properties of each individual (as far as they are revealed by its phenotype), 2) the quality of the transmission of hereditary information (as far as its disturbances are manifested in phenotypes), and 3) the quality of the transformation of hereditary information in individual development (which directly determines the properties of the phenotype).

Control of phenotypes is a necessary premise in the evolutionary transformation of the information. The control, i.e., the struggle for life, is realized within the system of the biogeocoenose by the confrontation of the activity of individuals with the available means for their existence and with other environmental factors. Transformation, i.e., natural selection, proceeds within the population itself on the basis of relative evaluation or testing of different variants in this confrontation.

Both the control and natural selection connected with it proceed throughout the life of every individual. The level of general life activity, almost always giving an individual certain advantages, is of basic importance in all testing. The forms of this activity, however, may be different and may be differently evaluated under different conditions. Natural selection under different conditions of existence takes various directions, and these depend both on the state of the biogeocoenose and on the particular properties and requirements of the population (Schmalhausen, 1938, 1939, 1941, 1946).

All of the mechanism of natural selection can be expressed in terms of information theory as the transformation of feedback information, transmitted by the phenotypes at the level of organization of whole individuals, into hereditary information, transmitted at the molecular level of chromosome organization. In this process, adjustments are made in the hereditary structure of the population on the basis of a comparative evaluation of individuals in the biogeocoenose.

Transformation of the hereditary structure of a population under sexual reproduction only starts with natural selection. It continues by the redistribution of hereditary information at gametogenesis and culminates in a new combination at fertilization and the evolutionary transformation of information is expressed in all of this.

Amount of Phenotypic Information and the Rate of Natural Selection in a Population of Haploids

The amount of information is a measure of the diversity of form. This diversity serves as the basis of natural selection and, apparently, somehow determines its potentialities. Since the amount of average information is given by simple summation of the average information brought in by each variant, the regularities of changes in it can be established from the individual variants and compared with the regularities of changes in the rate of natural selection (Schmalhausen, 1960).

For the simplest case, two alternative phenotypes must be considered for haploids (or gametes), while for diploids, the distribution involves three (or two, when dominance is complete) phenotypes, in accordance with the Hardy-Weinberg formula.

A maximum amount of information indicates an unstable state of the population, always ready to change its composi-

tion. The concepts of the amount of average information and that of the entropy level as the measure of degree of indeterminacy are thus related. In information theory the concept of entropy of distribution is used along with that of the amount of average information. Applied to populations, entropy is maximal when the choice between phenotypic variants is completely free. When comparing two alternative variants, both the amount of average information and the entropy level are at a maximum at the frequency of $p = 0.5$ for each variant.

Full freedom of natural selection when two alternative phenotypes are involved is provided when they have equal frequencies and equal selective merit. When this situation occurs, the population is in equilibrium, a condition not characteristic of the dynamics of evolutionary transformations.

Actually, natural selection has a direction. This means restriction of freedom, i.e., the absence of complete randomness of choice due to the introduction of a new factor, a higher selective merit of one of the two variants compared. However, the concept of amount of information also includes the notion of specific value of each of the variants. The curve expressing the amount of average information contributed by one variant in relation to its frequency has a very characteristic asymmetric form: it rises steeply from zero to its maximal value which lies at $p = 0.368$, and then drops more slowly to zero at fixation (fig. 1). The asymmetry of the curve is connected with a value of the variant. It was already mentioned that this evaluation has also a biological meaning. This is clearly revealed when comparing the change of the amount of average information with the change of the rate of natural selection under the same conditions. For these purposes we need not go into the details of the rather complicated mathematical theory of natural selection evolved by Fisher, Haldane and Wright. It suffices to compare the rate of selection for a single non-overlapping generation (e.g., of an annual plant) with the amount of phenotypic information for the same generation.

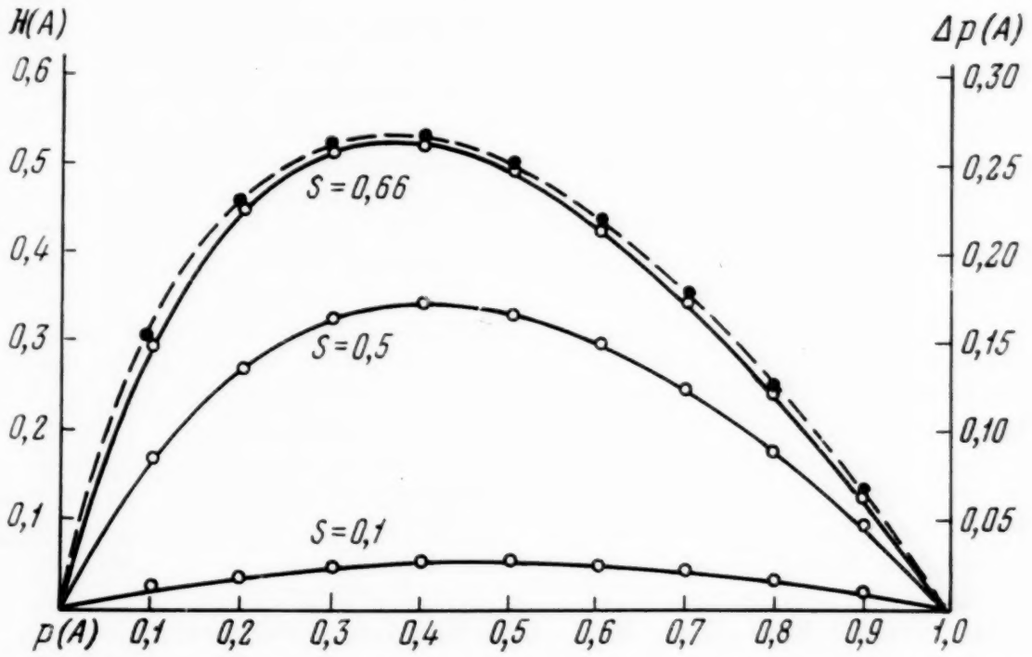

FIG. 1. Amount of average information H(A) and the rate of natural selection, ΔP(A), in relation to the frequency of the selected variant, p(A), at various values of selection coefficient S. The broken line indicates information amount.

If one of the alternative variants (A) leaves n progeny, while another (B), under the same conditions, leaves n (1-S) offspring, then S is the selection coefficient. Let us define the rate of selection as the increase in frequency of the favored variant per unit of time, i.e., per generation. Under these conditions the following simple formula gives quite exact results:

$$\Delta p = pq \frac{S}{1 - qS}$$

where p is the frequency of the favored variant, and q that of the other, with $p + q = 1$. With very small values of S, the fraction $\frac{S}{1 - qS}$ decreases, approaching in the limit the value of S. Then the above formula reduces to the well-known expression $\Delta p = pqS$ for low selection intensities. At the maximum value of $S = 1$, when one variant completely replaces the other, $\Delta p = q$.

The dependence of selection rate on the frequency of the favored variant is expressed by a curve which has almost a symmetrical form when the selection coefficient is low. In this case the freedom of choice prevails, i.e., either variant may be equally eliminated by chance. With the increase in the selective advantage of one of the variants (A), however, the curve of selection rate acquires an ever more asymmetric form. The maximum selection rate shifts toward lower concentrations the greater S is.

Both the asymmetry of the distribution of the amount of information, and the asymmetry of the distribution of selection rate in relation to the concentration of the selected variant are determined by the evaluation of the given variant. In the theory of information this value is expressed by the negative logarithm of the frequency of the given variant which decreases from indefinitely large values to zero when it is fixed in the population. In our formula for the rate of natural selection the value is given by another quantity, $\frac{qS}{1 - qS}$, which decreases with the increase in frequency of the selected variants from $\frac{S}{1 - S}$ to 0. At $S = 1$, it goes from ∞ to 0. At low values of S this decrease approaches linearity between S and zero.

The decrease in the selective merit of the variant with its increase in frequency has a deep biological significance. As in the theory of information, the merit of the variant is relative to that of the alternative variant (or more exactly, when considering different variants, relative to the whole population). If the variant A is very rare, then the individuals A compete throughout their life almost exclusively with individuals B and hence manifest their advantages. When A becomes frequent, it competes not only with B's, but with A's as well. In the latter case A has no advantage. At the frequency of A and B of 0.5, an A individual encounters individuals A and B equally frequently. Therefore A has lost the half of its advantage. At fixation, when the population consists only of A individuals, the variant A has no advantage with respect to other individuals of the given population. As in the theory of information, the advantage of a variant is evaluated on the basis of its relative frequency. However, the biological evaluation in the theory of natural selection differs from the mathematical evaluation in the theory of information not only in the manner of measurement, but also in the selection coefficient which characterizes the relationship between the two variants (A and B) in the biogeocoenose.

The selection coefficient, of course, expresses the difference between variants in the quality of information evaluated in the biogeocoenose. This evaluation is also relative in nature and leads to differences in frequency and, therefore, in the amount of information, in the next generation. Hence, the value of the selection coefficient can be determined not only by com-

paring frequencies of the favored variant in successive generations: ($S = \frac{p_1 - p}{p_1 - pp_1}$, where p_1 is the frequency of the selected variant in the next generation), but also by the amount of total (I) and average (H) information ($S = \frac{H_1 I - H I_1}{H_1 I - H H_1}$, where I_1 and H_1 are the amounts of information brought by the favored variant into the next generation). It follows from this that the rate of natural selection can be expressed entirely in terms of information theory

$$\Delta p = p_1 q s = \frac{H_1 I - H I_1}{I I_1 - H I_1}\left(1 - \frac{H}{I}\right).$$

High quality of information, and a high value of the selection coefficient ensures not only maximal rate of natural selection but, as we shall see now, also maximal utilization of the information available. We shall, however, restrict ourselves here to the consideration of the circumstances and extent to which the amount of average information by a given variant in a given generation actually restricts the possibilities of natural selection.

Under conditions of severe selection the biological evaluation of a variant approaches its quantitative evaluation in the theory of information. Then, the curve expressing the dependence of the rate of natural selection on the frequency of the favored variant approaches the curve expressing the amount of average information. At $S = 0.66$, the two curves (when reduced to comparable scales, i.e., the one for selection rate being double the other) nearly coincide, the maximal height of both curves (when logarithms are to the base 2) being at $p = 0.368$. Numerically, the rate of selection reaches half the amount of average information. Apparently, under severe selection, the amount of information already restricts its rate. It is natural that it is under severe selection, that its rate is limited by the amount of corresponding material in the population.

Under natural conditions of existence of a population such severe selection apparently does not occur. The selective advantage in directed selection cannot be large. The favored variant cannot exceed the average in all traits at once, even when a sharp change of conditions in the biogeocoenose takes place. Under stabilizing selection, selective advantage can be very large and the selection coefficients can reach its maximal value of 1 (elimination of lethals). Here the maximum selection rate obtains only at high frequencies of the variant eliminated. Such frequencies are impossible under natural conditions, since each population can exist only when the "normal" types are predominant in number, and not when strongly undesirable variants are prevalent. At higher values of S, the rate of stabilizing selection is limited by the quantity of eliminated material (it approaches $\Delta p = q$) and, eventually, by the rate of origin of a given mutation. In any case, these rates are low.

In our discussion, frequency has been expressed as usual in the form of a fraction giving the proportion of the variants in the population, and increasing linearly from 0 to 1. It is evident that each successive increase in this value is of lesser significance to the whole population than the preceding one. Hence it would, perhaps, be more correct to measure the frequency of a variant by the logarithm of the generally accepted fraction. The logarithms to the base 2 are particularly suitable in this case, since each increase of this value by a unit indicates doubling of frequency. Selection rate then can be expressed as the increment in the logarithm of frequency per unit time, i.e., per generation. In this case, $\Delta \log p = -\log (1 - qS)$. Some regularities are particularly clearly revealed by using this measure of selection rate.

In the limit, at $S = 1$, logarithmic rate of selection equals the total amount of information, as $1 - qS$ in this case gives $1 - q = p$, therefore $\Delta \log p = -\log p = I(A)$. This implies that the informa-

tion I(A) fully determines the rate of natural selection at its maximal value. At $S < 1$, information is not completely utilized, while at decreasing values of S, the logarithmic selection rate depends to an ever-increasing extent on the frequency of the eliminated variant (expressed in the usual form). At $S = 0.5$, the logarithmic selection rate is already almost proportional to the frequency q. At relatively low values of the selection coefficient, i.e., in all real cases of the directed form of natural selection, the logarithmic rate of selection A is proportional to the frequency q of B, i.e., it decreases proportionally to the decrease in the probability of encounters between a given A with any B.

Amount of Phenotypical Information and the Rate of Natural Selection in a Population of Diploids

If in a population of haploids the proportions of the alternative types A and B are determined by the formula $p + q = 1$, in an equilibrium population of diploids they are determined by the formula $(p + q)^2 = 1$, or $p^2 + 2pq + q^2 = 1$. In this case there are three genotypes, AA, Aa and aa, each with its corresponding phenotype. If we represent graphically the change of the amount of average information in relation to the frequency of a given phenotype, we would obtain the same curve as in the case of haploids. However, the frequency of one of the phenotypes, Aa, changes with the change of the freqency of AA or aa in a non-linear fashion: at first it increases up to the maximum of 50% of the population, and then decreases to zero, when one of the homozygotes becomes fixed. As a result, the concentration of the heterozygote Aa twice reaches the level of 0.368, corresponding to the maximum amount of information. Hence, the curve has two maxima.

The rate of natural selection of all the three phenotypes is determined by their selective advantages, expressed by the coefficient S for one of the homozygotes and coefficient hS for the heterozygote. The value h is an index of dominance. Under complete dominance, $h = 0$, and the phenotype Aa is not distinguishable from

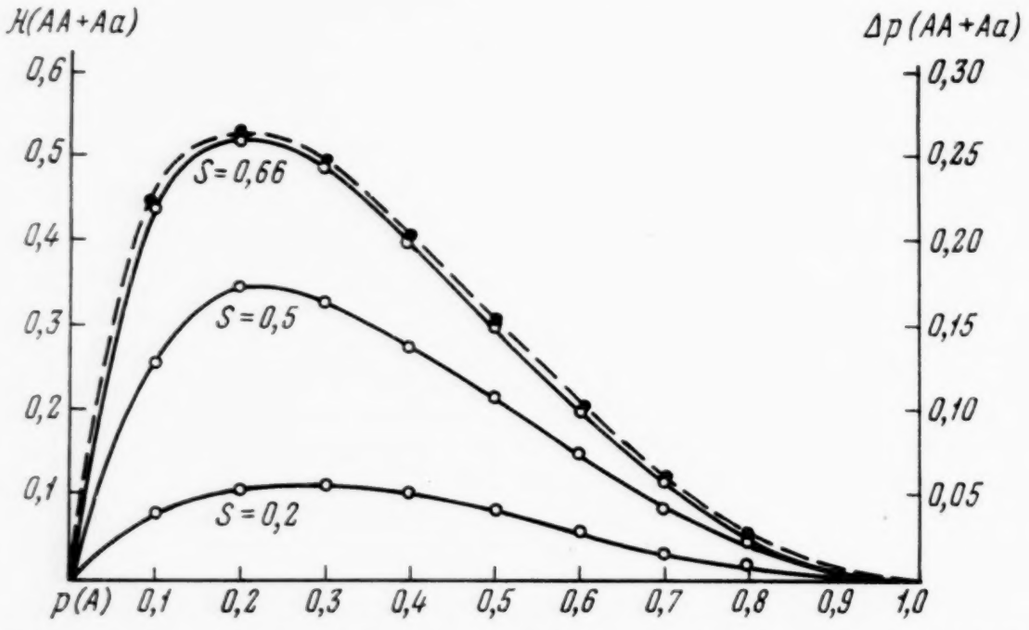

Fig. 2. Amount of average information, H(AA + Aa), and the rate of natural selection, Δp(AA + Aa), of the dominant phenotype in relation to the frequency of the selected gene, p(A), at S = 0.2, at S = 0.5 and S = 0.66. The broken line indicates amount of information.

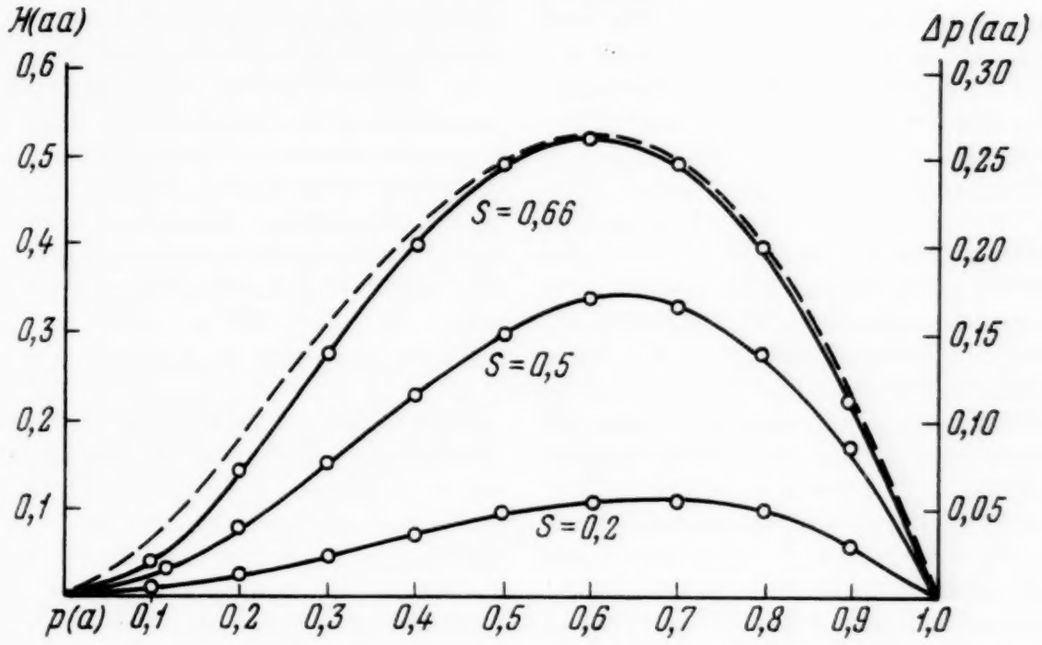

FIG. 3. Amount of average information, H(aa), and the rate of natural selection, Δp(aa), of the recessive phenotype in relation to the frequency of the selected gene, p(a), at S = 0.2, at S = 0.5 and S = 0.66.

AA, so that again only two alternative phenotypes (AA + Aa and aa) are present, though in a different ratio than before. Both the amount of average information and the rate of natural selection are determined by such ratios.

In the theory of natural selection we are interested mainly in hereditary changes, so that the rate of natural selection, based on phenotypes, is considered in terms of frequency changes in the hereditary units, the genes. Here, the distribution of selection rates seem to be characteristically different for the dominant and recessive phenotypes (figs. 2 and 3). This difference, however, is only the expression of numerical ratios of phenotypes in the case of complete dominance. That is why this difference is to an equal extent expressed also in the distribution of the average amount of information. The curve showing the change in the rate of natural selection in relation to the freqency of corresponding genes preserves its general similiarity to the distribution curves of the amount of phenotypical information. The curves approach each other under severe selection. At S = 0.66 they almost coincide, while the rate of natural selection reaches, in the numerical expression, half of the amount of information for the given phenotypes.

The curves showing the amount of average information and the rate of natural selection of the heterozygote (fig. 4) have, as mentioned, two maxima which coincide at S = 0.66. The curve of selection rate of Aa is interesting not only because of its shape. It also demonstrates very favorable conditions of natural selection of the heterozygote within the wide range of concentrations of either of the alleles from p = 0.1 up to p = 0.9, or of that of each of the homozygotes from p = 0.01 up to p = 0.8. The peculiarity of these curves depends on the evaluation of the phenotype in the theory of information as well as in that of natural selection. In both cases the phenotype is evaluated not by its own properties but by its advantage over the alternative variant. In the case of the heterozygote the evaluation has two maxima: one against the background of absolute numerical prevalence of the homozygote aa, and the other one against the background of absolute preva-

lence of the homozygote AA. The first maximum is determined by the probability of encounter between individuals Aa and aa, while the second one is determined by the probability of Aa to encountering AA.

These bimodal curves of the amount of average information and the rate of heterozygote selection clearly show that the purely mathematical evaluation of the variants by their distribution has also a biological meaning: it characterizes the position of the variant in a population and shows the possible distribution of selection rates. However, as already stated, actual selection rates are determined not only by the position of the variant in the population, but also by its position in the biogeocoenose which is characterized by the selection coefficient S, i.e., by the quality of information evaluated in the biogeocoenose.

In haploid organisms, natural selection based on phenotypes is at the same time selection of genotypes which leads to changes of gene frequency in the population to an equal degree. Diploidy leads to the existence of an intermediate type which can play a different part in selection. Under complete dominance, the rate of selection of a dominant gene decreases due to the selection of Aa's which contain, along with the selected gene A, also the eliminated gene a. The rate of selection of a recessive gene is also lower since it is not selected for when in an Aa phenotype.

Conclusions and Summary

Application of general principles of cybernetics and information theory allows one to build up a simple scheme for the elementary cycle of evolutionary transformations of population with a clear differentiation of the main steps of this cycle. New conceptions allow more exact formulations, providing a possibility of further analysis and the application of new methods of quantitative evaluation of phenomena. The new viewpoint makes more understandable a number of points:

1. The complex organization of the hereditary code and the necessity of the exact transmission of hereditary informa-

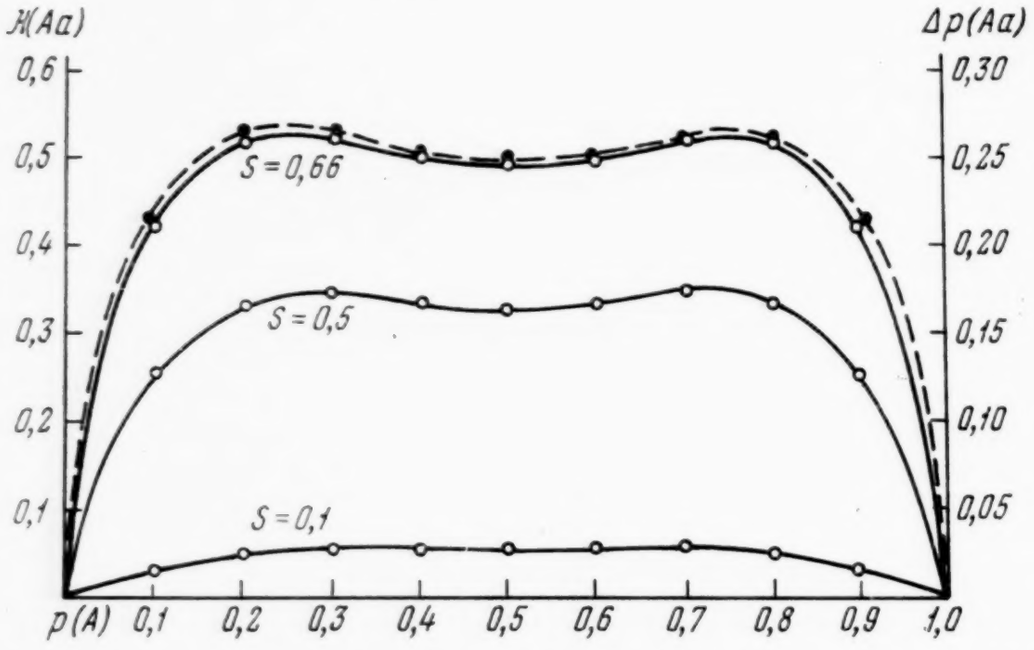

Fig. 4. Amount of average information, H(Aa), and the rate of natural selection, Δp(Aa), of the heterozygous phenotype in relation to the gene frequency, p(A), at S = 0.1, at S = 0.5 and S = 0.66. The amount of average information is designated by the broken line.

tion; protection of this mechanism against disturbing influences of accidental external factors (noises).

2. The need for a stable, perhaps, autonomous mechanism of transformation of information in individual development.

3. The significance of adequate phenotypical information as a condition for control in the biogeocoenose of both the transformations in the hereditary code, and the evolution of individual development.

4. The significance of external factors as the means of control of characters of the phenotype and the division of these factors into indispensable ones, i.e., means for existence, and accidental, more or less adverse ones, i.e., noises.

5. The role of population structure and of the position of the individual variants in it, in the possibilities for effective action of natural selection.

The introduction of new methods of quantitative evaluation of the diversity of forms in populations also allows one to judge more exactly their general organization (entropy level) and their evolutionary plasticity. The validity of these methods is proved by the statement of relation between the amount of phenotypical information and the rate of natural selection at its upper limit.

To reiterate, analysis of evolution from the viewpoint of cybernetics permits one to divide the elementary cycle of evolutionary transformations of population into separate steps, to distinguish the factors responsible for these transformations, and to introduce new methods of quantitative evaluation of the phenomena taken under study. The elementary cycle of evolutionary transformations consists of 1) the transmission of the direct, hereditary information through the reproductive elements of each generation, 2) transformation of this information in the individual development of the phenotype, 3) transmission of the feedback, phenotypical information, 4) control of phenotypes by means of their comparative evaluation in the biogeocoenose, and 5) transformation of hereditary information through natural selection (and the sexual process) within a given population.

The basis of the transmission of hereditary information is the copying of its code in the self-reproduction of chromosomes. The possibility of such replication is determined by the structure of the DNA molecule allowing its splitting with subsequent restoration. Alternation of transverse bonds of adenine with thymine or of guanine with cytosine plays the role of primary information elements determining the individuality of biological information units, the genes. Information reliability is ensured by means of coupling these units into long blocks, the chromosomes, as well as by means of the repetition of some genes and entire chromosomes (in diploid or polyploid organisms). Noise resistance is ensured also by the insulation of the hereditary code and by its protection by regulatory mechanisms of the cell and of the whole organism. The sum of negative logarithms of frequencies of particular genes multiplied by their respective frequencies provides the measure of the amount of average hereditary information per gene in the population,

$$H = -\Sigma\, p_i \log p_i.$$

Not only does the transmission of hereditary information to all cells take place throughout individual development, but there occurs also its transformation into the system of the developing soma, and its realization in the characters of phenotype. The phenotype of an individual is an indivisible unit of feedback information from a population to the biogeocoenose. Transmission reliability of the feedback information is ensured by the organization of the population, the reiteration of all its important elements in each individual, and by the system of life protection of each individual. The amount of phenotypical information in a population (or average information per individual) is estimated from the sum of negative loga-

rithms of the frequency of each variant, multiplied by its respective frequency.

The phenotype of an individual is the object of control in the biogeocoenose (struggle for existence). External factors (indispensable, means for existence, and accidental, noises) are the means of control. The control of phenotypes is the basis of natural selection. Natural selection, supplemented usually by the sexual process, plays the role of a transformation mechanism of phenotypical information transmitted at the level of the organization of an individual into hereditary information transmitted at the level of chromosome organization to the zygotes of the next generation.

Since the phenotypical diversity of forms is the material basis of natural selection, certain relations exist between the amount of information, characterizing this diversity, and natural selection. Under severe selection, the curve expressing its rate in relation to the frequency of selected variants is very similar to the curve for the amount of average information on the same variant. The rate of selection is clearly limited by the amount of phenotypical information. Both the amount of information and the rate of natural selection are determined by the evaluation of a given variant relative to the alternative variant. The rate of natural selection, however, also depends on the position of variants in biogeocoenose characterized by the selection coefficient. The latter is the expression of difference between two variants in the quality of information. High quality of information, and high values of the selection coefficient, ensure the maximal utilization of information and maximal rates of natural selection.

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NOTES AND COMMENTS

MATING PREFERENCES IN *DROSOPHILA*

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The recent paper by Hoenigsberg and Koref Santibanez (EVOLUTION 14: 1–7) on courtship and sensory preferences in *Drosophila* merits further discussion because in the interpretation of their data, they repeatedly refer to the "preferences of the males." This interpretation undoubtedly stems from the experimental method employed, the so-called "male choice" method. Though widely used in work on sexual preferences and sexual isolation in *Drosophila*, it is an unfortunate choice because the best available evidence indicates that within a species or with closely related species, male *Drosophila* show little if any sexual preference. Streisinger (1948) showed, for example, that male *melanogaster* mated at random with etherized *melanogaster* and *persimilis* females, but when placed with unetherized females of both species, the *melanogaster* males succeeded in mating only with *melanogaster* females. Comparable results were obtained with *pseudoobscura* and *persimilis*. Working with unetherized flies of the latter two species, Merrell (1954) showed an isolation estimate of .069 in female "choice" experiments and .059 with multiple "choice" (zero equivalent to complete isolation), but a much lower degree of isolation (.457) with male "choice" experiments. Work with various mutants of *melanogaster* (Merrell 1949a) showed that deviations from random mating were much greater and occurred more often in female "choice" than in male "choice" experiments. In fact, the deviations from random mating in the male "choice" tests appear to be due to the behavior of the females rather than any preference on the part of the males. Furthermore, Merrell (1949b) has shown that the duration of courtship appears to be determined by the type of female. This evidence seems to suggest that male *Drosophila* have a low level of discrimination as compared to the females, and that female "choice" tests are much more sensitive than the widely used male "choice" method.

However, even the male "choice" method can yield significant results, particularly if it is realized that these results may not, in fact, be due to any choice on the part of the males. Data on mating behavior have shown that two distinct phenomena may be involved, preferential mating and sexual isolation. The data must be examined from both viewpoints lest in experiments on sexual isolation, for example, isolation may appear to exist where in fact it does not, and the spurious positive result is due to selective mating. Simple tests have been devised (Merrell 1950) for disentangling these effects. With these factors in mind the data of Hoenigsberg and Koref Santibanez have been re-examined and I, M_f, and M_m have been calculated.

It may be seen in table 1 (part A) that the significant fact in regard to the number of copulations is not that "the Samarkand strain shows a significant tendency toward homogamic matings," but rather that Samarkand females mated more than twice as often as Oregon females ($M_f = 2.25$) with either type of male, and no real tendency for homogamic mating does, in fact, exist ($I = .86$). With tapping, however, a real homogamic tendency does appear ($I = .56$), but even more striking is the fact that Samarkand males tap only one third as often as Oregon males ($M_m = .34$).

The apparent "significant preference for copulation with their own females" by Oregon outbred males (part B of the table) is attributable to the fact that the inbred females mated much less frequently than outbred females ($M_f = .59$) with either male type. Hence male preference is not involved.

Part C of table 1 deals with "courtship preferences." In all cases the time spent courting the outbred females was less than that spent courting the inbred females (M_f ranged from .75 down to .24). The males, on the other hand, varied in sexual drive. While outbred Samarkand and Oregon males were high compared to Oregon inbred 13 ($M_m = 1.83$ and 1.43), they were low compared with Oregon inbred 14 ($M_m = .69$ and .74). The data of parts B and C seem to suggest that here too the female plays the more significant role. The inbred females, which mate less often, appear to require a longer courtship than the outbred females. In view of the fact that the outbred males spent equal or greater amounts of time courting the inbred females than they did courting their own females, these data cannot be said to indicate incipient sexual isolation, and the low I values are spurious.

The orientations recorded in part D and the tappings in part E show a pattern similar to Part C, in that in all cases the displays are less often directed toward the outbred females than toward the inbred females. It is of special

TABLE 1. *Courtship behavior statistics computed from tables 1–5 of Hoenigsberg and Koref Santibanez (1960)*

Part	Behavior trait	Isolation index I	Female mating ratio M_f	Male mating ratio M_m	M_f and M_m Numerator/Denominator
A	Courtship time	1.15	.73	1.18	Samarkand/Oregon
	Copulation number	.86	2.25**	1.09	Samarkand/Oregon
	Tapping	.56**[a]	.79	.34**	Samarkand/Oregon
	Orientation	.83	1.19	.79	Samarkand/Oregon
B	Copulation number	.78*	.59**	.79	Oregon inbred/Oregon outbred
C	Courtship preference	.88	.75**	1.83**	Samarkand/Oregon inbred ♀
		.35**	.42**	.69**	Samarkand/Oregon inbred ♂
		.57**	.33**	.76**	Samarkand/Oregon inbred ♀
		.69**	.50**	1.43**	Oregon/Oregon inbred ♀
		.65**	.24**	.74**	Oregon/Oregon inbred ♂
		.67**	.60**	1.06	Oregon/Oregon inbred ♀
D	Orientation	.58**[a]	.84	.75**	Samarkand/Oregon inbred ♀
		.50**[a]	.68**	.43**	Samarkand/Oregon inbred ♂
		.71**	.82*	.84	Samarkand/Oregon inbred ♀
		.66**	.70**	.90	Oregon/Oregon inbred ♀
		.80**	.60**	1.14	Oregon/Oregon inbred ♂
		1.06	.68**	.66**	Oregon/Oregon inbred ♀
E	Tapping	.60**[a]	.80	.72**	Samarkand/Oregon inbred ♀
		.44**[a]	.65**	.47**	Samarkand/Oregon inbred ♂
		.52**[a]	.81	1.14	Samarkand/Oregon inbred ♀
		.64**	.66**	.88	Oregon/Oregon inbred ♀
		.85	.57**	1.14	Oregon/Oregon inbred ♂
		.64**	.81	.91	Oregon/Oregon inbred ♀

* Significant. ** Highly significant.
[a] Data indicate sexual isolation.

interest that two I values in part D and three in part E appear to indicate a significant degree of sexual isolation between the strains involved.

In conclusion, the "copulatory preference by outbred males" (part B) appears due in reality to the lower frequency of mating by inbred females. The constant factor in the last three parts of the table is the fact that inbred females were courted more frequently than outbred females by both inbred and outbred males. To refer to this behavior as due to "male preference" can be misleading in view of the evidence cited earlier that *Drosophila* males show little or no preference in mating. The differences observed may have been due merely to the fact that the inbred females moved about less than the outbred females or to some other aspect of female behavior. Most significant is the demonstration that some sexual isolation has developed between these strains. Use of the female "choice" method might have provided a more sensitive test of the degree of isolation.

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SEXUAL BEHAVIOR: A DISCUSSION

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Merrell's main criticism in the preceding note refers to the use of the "male choice" method. He claims that studies by Streisinger (1948) showed that males exhibit almost no sexual preference, and that such preferences should be ascribed to the females. He uses the isolation estimate method and the female and male mating ratios to demonstrate that in many instances there is no evidence of courtship tendencies as claimed by the χ^2 method used in our paper. Merrell's criticism is based on the definite assumption that the female choice method might have provided a more sensitive test of the degree of isolation. The differences observed in homogamic versus heterogamic tendencies, he says, may be due to the fact that the inbred females moved about less than the outbred females. However, we believe that the incipient sexual isolation shown in our results is due to other aspects of female behavior. We said in our paper that female discrimination has to be recognized as the main factor in determining sexual preference, although the male contribution to discrimination is also evident. In our view, both sexes determine the "choice" involved in discriminating courtship, but we do not have the evidence to establish the relative importance of the two let alone to indicate complete lack of the importance of the male.

The data presented by Streisinger (1948) refer to the behavior of males in copulations and not in courtship, and toward etherized females rather than normal females. The females, not having any threshold barrier, were equally neutral to the attacks of the males, who simply mounted and copulated with them. It is, however, in the preparatory phases of courtship that the importance of female behavior enters. The first elements of courtship, such as orientations and tappings, may be indiscriminatory in some cases, while the latter steps in courtship exhibit an extensive priority in "choice" on the part of the male, even if copulations do not necessarily follow the indicated trend of courtship (Hoenigsberg and Koref Santibanez, 1959b and 1960b). The inbred females have to be non-etherized in order to make evident threshold responses toward both males, thereby stimulating and promoting preferential behavior in one of them. Data on lickings, standings, circlings, wing vibrations, courtship time, etc., not presented in the relatively short paper which appeared in EVOLUTION have been published elsewhere (Hoenigsberg and Koref Santibanez, 1959a, b; Hoenigsberg, Koref Santibanez and Sironi, 1959). They presented considerable further information on female and male behavior and the importance played by some elements of courtship. From our experience we can say that 1) not all elements of courtship elicit response of the same intensity, (this fact must be of great significance in the lowering of the female threshold), and 2) that courtship time of females should be an indication of their critical ability to maintain the threshold.

Furthermore, while studying intraspecific courtship discriminations in *D. aequinoctiales* and *D. prosaltans*, we found that only some Colombian and Brazilian males exhibited preferences in courtship time, tappings, lickings and standings of the females, while the other tropical American strains from Costa Rica, Cuba, and Colombia did not. The evolutionary significance of discriminatory behavior found in those two species is discussed by us elsewhere (Hoenigsberg and Koref Santibanez, 1960b).

Obviously the problem of incipient sexual isolation is not solved by merely deciding which sex is the discriminating partner in courtship, since probably both display preferences, but calls for a detailed analysis of courtship patterns. The behavior which we discussed in our paper involves sensory discrimination in general rather than male preference only. The name "male choice" was used in the absence of a better term, only to describe the method designed by Dobzhansky and Mayr (1944).

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COMMENTS ON THE ORIGIN OF THE AMNIOTE EGG

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In a recent discussion of the origin of the amniote egg, Romer (1957) expresses the belief that the earliest reptiles were amphibious or semi-aquatic, as were their immediate amphibian ancestors. The amniote egg, one of the major achievements of tetrapod evolution, was developed by such semi-aquatic animals, not by a group of animals in which the adults had already become terrestrial. As he states it: ". . . although the terrestrial egg-laying habit evolved at the beginning of reptilian evolution, adult reptiles at that stage were still essentially aquatic forms, and many remained aquatic or amphibious long after the amniote egg opened up to them the full potentialities of terrestrial existence. It was the egg which came ashore first; the adult followed."

This thesis is logically developed and eminently reasonable. But the further speculations concerning ecological conditions immediately circumstant to the development of the amniote egg are perhaps more open to question. Certainly, as he pointed out, the terrestrial egg was developed as a matter of immediate advantage, not through any far-sighted "urge" towards a terrestrial existence. Moreover, it is entirely reasonable to assume that this advantage lay in avoiding the necessity for an aquatic existence during the particularly vulnerable immature stages of the life history. But that the advantage arose originally because of seasonal or intermittent drouth conditions, with a consequent uncertain supply of water and concomitant danger of desiccation, is questionable. Inger (1957), in discussing the origin of tetrapods, stated that "it should be possible to judge any hypothesis on the basis of current knowledge of the ecology (in the broadest sense) of living vertebrates, geochemistry, and paleoecology." The same criteria apply here.

When properly interpreted, data from geochemistry and paleoecology provide the most direct evidence obtainable concerning the conditions under which any given fossil form or group lived, provided that these data are acquired from the deposits directly involved, not from some broad generalization about "extensive areas of tropical swamps," "generally cool," or others of a similar nature. As Dunbar (1949) points out: "It is well to remember that at present the dripping jungles of the Amazon valley are separated only by the narrow Andean chain from the desert coast of western Peru." For evidence that temporal changes within a given area can be equally drastic and abrupt, one needs look only to the Pleistocene.

It is also necessary to recognize that the earliest ***known*** representatives of a particular group are unlikely to be actually the earliest members of that group. Conditions indicated by the deposits in which the earliest known specimens are found do not necessarily reflect the actual conditions surrounding the origin of the group. Nevertheless, the nearer to its actual origin that we can trace a group's history, the more likely it is that the earliest representatives are living under conditions similar to those surrounding its origin.

The earliest known reptile remains seem to be those of *Cephalerpeton* from the Mazon Creek locality of Illinois. The fossiliferous beds lie near the base of the Carbondale formation, corresponding in age to the mid-Allegheny of the Appalachian region and probably the early upper Westphalian of Europe (Gregory, 1950). The reptilian nature of *Cephalerpeton* seems reasonably well established; if one does not accept this form as reptilian, then the next candidates for the title of "earliest known reptile" are from the cannel coal beds of Linton, Ohio or the "Gaskohle" of Nyrany, Bohemia. These are of upper Allegheny or uppermost Westphalian age. In any event, the fossils from all three localities are found in deposits that must have represented continuously humid tropical or subtropical swamps and that contain, in addition to the reptiles, a number of amphibians

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and some fish. For a more detailed discussion of ecological conditions and faunas, reference may be made to Gregory (*op. cit.*), Romer (1930) and Westoll (1944), and to earlier works cited by these authors.

It is true that in the Permian many reptiles are associated with deposits indicative of arid to semi-arid conditions or intermittent drouth. But to the best of my knowledge the only Pennsylvanian occurrence of this nature is that of *Petrolacosaurus* in shales near Garnett, Kansas (Peabody, 1952). These shales are correlated with the Upper Conemaugh of the Appalachian region and the middle Stephanian of Europe, thus are decidely younger than the deposits previously discussed.

Because of the limitations already mentioned, and the accidents of geological preservation, these data admittedly do not provide conclusive evidence that the reptiles first arose in a coal swamp environment. But they provide even less evidence for any other interpretation. The simple fact that ". . . large areas of the earth in late Paleozoic days appear to have been subject to marked seasonal drouth . . ." can hardly be considered as evidence that the amniote egg appeared under drouth conditions. It is only in the Upper Pennsylvanian and Permian that reptiles are *known* to be associated with such climatic and environmental circumstances; these more arid conditions were almost certainly a major factor in the diversification and spread of reptiles, but there is no direct evidence that they played any part in the origin of the class.

Of greater significance are the inferences that may be drawn from our knowledge of the ecology of living amphibians. Many of these lay eggs out of water. The few salamanders with terrestrial eggs include several plethodontids, the amphiumids and *Ambystoma opacum*. Many anurans have non-aquatic eggs, as do coecilians; reference may be made to Noble (1931) or Angel (1947) for a listing of examples and a discussion of some of the mechanisms employed. Certain significant generalizations emerge from an examination of these amphibians and their eggs. Firstly, the non-aquatic development is often confined to the eggs; a normal aquatic larval stage still occurs. In many instances of this sort the egg deposition might more properly be described as "beside" the water rather than "outside" of the water. Completion of development requires immediate access, in one way or another, to a thoroughly dependable and permanent supply of water; the non-aquatic existence is confined to the earliest, most vulnerable stages of development. Secondly, none of these eggs exhibit any obvious structural or physiological modifications that are clearly interpretable as devices to reduce the danger of desiccation. Desiccation is avoided by behavioral mechanisms, including choice of site of deposition and others, that insure an adequate supply of moisture in the immediate vicinity of the eggs. Thirdly, perhaps simply one aspect of the second point, the amphibians having terrestrial eggs, with only rare exceptions, live in regions of high humidity, with abundant and permanent water readily available for egg deposition and larval growth. Even those few living in somewhat more arid circumstances deposit the eggs in situations of essentially permanent moisture.

On the other hand, many amphibians do today live in regions where the water supply is intermittent and undependable. These simply make more efficient use of such water as is available; many, for example the spadefoot toads, have responded to the uncertainty of water supply by a reduction of the length of time required for development of the egg and tadpole to the metamorphosed adult form, but this development remains purely aquatic. I know of no examples of mechanisms or structures developed in such forms that could be interpreted as "desiccation-avoiding" features.

It seems obvious then that the terrestrial eggs of modern amphibians are a device for escaping predation in the most vulnerable stages of the life history, and probably in some instances for assuring an adequate oxygen supply for the developing egg, not a device for avoiding desiccation. Romer recognizes this, but postulates that a reverse situation existed in the Paleozoic, that "potential egg devourers were then presumably less abundant, but danger of desiccation was far greater." This is very unlikely. Perhaps potential egg devourers were less abundant in total number, or total number of kinds, but the *differential* in predation pressure between water and land must have been far greater at that time. Pennsylvanian ponds supported an abundant vertebrate and invertebrate life; many of these forms must have developed a taste for amphibian eggs. But the terrestrial fauna was much more limited than at present, especially with respect to vertebrates. This does not imply that terrestrial predation would not occur at all, but simply that if there is, as appears, an advantage now in removing the early, predation-vulnerable stages out of the reach of aquatic predators, such an advantage must have been of far greater magnitude in the Paleozoic. Furthermore the coal-swamp habitats of the Pennsylvanian are thought to have been, on the whole, very low in oxygen. Eggs deposited beside or outside of the water so as to utilize aerial oxygen would be able to develop more rapidly; the length of the predation-vulnerable period would thus be notably decreased.

This all demonstrates that very strong selection pressures favoring a terrestrial egg

can and do exist even when abundant water is available. One need not postulate intermittent drouth conditions to demonstrate a selective value. In fact, as judged from recent examples, danger of desiccation leads only to the deposition of eggs more positively within, rather than on the fringes of, the bodies of water available; danger of desiccation is far greater in any marginal niche. One would be inclined to postulate development of the amniote egg under conditions of abundant moisture simply because in the early stages of its evolution it must have been quite susceptible to desiccation. Only after the specializations that now protect it had been developed could it be situated in even moderately dry surroundings. Even the reptile eggs of today, for the most part, require a certain minimum moisture for development. Truly, many develop under conditions completely intolerable for any amphibian egg, but the early imperfect amniote egg surely required abundant moisture. It would require a rather delicate balance of wet and dry seasons to produce a condition in which there was insufficient water for aquatic development throughout the egg and larval stages, yet a sufficient amount of mud or a similarly moist medium that this delicate early egg would survive.

A further argument in opposition to the idea that intermittent drouth was a major factor in the development of the amniote egg, and in support of the contention that it arose under continuously humid, perhaps "rain-forest" conditions, is the conclusion of Romer that the earliest reptiles were still semi-aquatic in the adult stage. To develop the terrestrial egg from such adults, we are asked to postulate conditions such that there was a sufficiently dependable water supply to allow the survival of these semi-aquatic adult animals, yet so uncertain a supply that the eggs and young were in danger of desiccation if they remained aquatic. There appears to be an inherent inconsistency in such a postulation.

One final purely theoretical consideration might be mentioned. Almost any major evolutionary "break-through" involves some general adaptation—a set of functionally related features that are advantageous to their possessors in a very wide range of environmental situations (cf. Darlington, 1957, and Smith, 1959). It is this general advantage that allows the extensive radiation of members of the new group into a multitude of habitats, including those previously occupied by the ancestral group, and the consequent recognition of a major systematic category. It follows that these "new" features would confer a selective advantage in virtually any environmental situation in which they might happen to appear, and would become established and expanded regardless (within reasonable limits) of what that environment might be.

If this is the case, the critical condition for the establishment of a new group is not the presence of any unusual or stringent environmental conditions, but the fortuitous occurrence of some rather extraordinary genotype in *any* environmental situation. The chance of getting the "right" genotype would presumably be correlated directly with the total amount of genetic variability and genetic potential on which the machinations of probability could operate. This greatest total variability and potential would, in turn, be found wherever the total number of individuals and, more importantly, of species, is highest. Occurrence of a large number of species and of individuals implies environmental conditions extremely favorable to the ancestral group. As Smith (*op. cit.*) states, summarizing Darlington's principles, "New groups . . . arise in areas most favorable for the maximum number of types, not in peripheral zones." A rigorous environment would tend very strongly to limit the possible number of genotypes that could survive in it; a favorable environment allows far greater genetic variability to be developed and maintained. But the general advantage offered by the new features, as envisoned above, would give their possessors a selective advantage over the ancestral stock even in the environment most favorable to that ancestral stock.

In the particular instance of the origin of the amniote egg, the existence of probable strong selective advantages in the environment favorable to the ancestral amphibians has been demonstrated. It appears that the greatest variety and number of Paleozoic amphibians lived in tropical or semitropical swamps. In just such areas, then, we would expect to find the greatest genetic potential, and the appearance of that genetic constitution that represented the establishment of the amniotes.

In summary, I believe that the simplest interpretation of the probable immediate circumstances surrounding the development of the amniote egg is that it was developed, as suggested by Romer, as the terrestrial egg of semi-aquatic adult animals, but that it appeared under very humid, probably swampy and tropical, climatic conditions.

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A FUNCTIONAL INDEX OF HYPSODONTY

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Hypsodonty, or relative increase in the height of a cheek tooth, is common in mammals (Simpson 1953, White 1959). The usual measure of this is the ratio of the height of the tooth to its length or width. Its functional significance is to permit a longer maximum life span for an animal of a given size, or to allow coarser and more abrasive food to be eaten without decreasing the life span. It is not uncommon in the fossil record to find individuals with the teeth worn nearly or quite to the roots, so greater hypsodonty is apparently of some selective value in populations where this situation occurs before complete reproductive senility.

Other changes in the tooth, however, also lead to the same adaptive consequences. These are increase in the area of enamel in occlusion (resulting from greater occlusal area of the tooth, thicker enamel, or greater complexity of the enamel pattern) and increase in the resistance of the enamel (also dentine and perhaps cement) to abrasion. Probably relevant changes outside the teeth, such as in jaw movement, also have occurred, but are more difficult to evaluate in terms of their effect on dental attrition. Other conceivable directly relevant trends, e.g. toward a greater ability to efficiently digest less chewed food, are speculative.

A quantitative comparison of the efficiency of cheek teeth within and between populations of an evolving phyletic line, and between related and unrelated phyletic lines, would be of considerable interest in various questions relating to the existence and nature of evolutionary progress. It would be of interest to know, e.g., whether hypsodonty (and the other aspects) are largely involved in the necessary compensation for increasing size, or whether (and how much) they are also progressive adaptations in themselves. The following proposal, in part analogous to but developed in ignorance of that of Watson (1949), is an attempt to arrive at a testable method for such comparisons.

The weight of an animal is approximately proportional to the cube of a linear dimension that adequately describes its size. This linear measurement should if possible not be made on the teeth, to avoid complications from the other components of the same adaptation. The metabolism of a mammal, however, and therefore presumably its food intake, is approximately proportional only to the ¾ power of its weight (Kleiber 1947; see also Watson 1949). Therefore, a rough estimate of the *relative* food intake may be taken as the square (or, more accurately, the 2¼ power) of an appropriately chosen linear measurement. This exponent is, of course, the product of ¾ and 3. The *relative* amount of food per unit tooth height would then be about

$$\frac{h}{m^2} \text{ or } \frac{h}{m^{2.25}},$$

where h is an appropriate measure of tooth height and m is a linear measure of the size of the animal (or the mean of any number of such linear measures). To compare two populations, or two individuals in a population, divide the above ratio for one by the ratio for the other. This will give an approximate index

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of the relative efficiency of hypsodonty in increasing the amount of possible attrition and therefore in prolonging the maximum life span and permitting more abrasive food to be eaten. The first of these two effects can in some cases be tested directly in both fossil and recent populations, but any test made should also include the other components in some such manner as described below.

Similar indices can be formulated, changing only the numerator of the first ratio, for the other components of abrasion resistance mentioned above. The numerators should be multiplied together and this product divided by m^3 or $m^{2.25}$ to obtain a rough estimate of the total relative efficiency of the teeth in resisting attrition. For example, if l is an adequate measure of the mean total length of enamel exposed at each stage of wear, t is a measure of the mean thickness of the enamel, r is a measure of the abrasion resistance of the enamel, and the subscripts 1 and 2 refer to two related populations, the index of comparison would be

$$\frac{\frac{h_1 l_1 t_1 r_1}{m_1^{2.25}}}{\frac{h_2 l_2 t_2 r_2}{m_2^{2.25}}}.$$

If one or more of these items does not differ measurably between the two populations, it can be eliminated from the computation. It may be desirable, in comparing several populations, to use one of them always in the denominator and thus compare all the populations with each other directly. It is possible that hypsodonty may be greater in a small animal if there is proportionally less occlusal area of the cheek teeth (or the animal lives longer, etc.). In mammals with truly ever-growing teeth, h will be indefinitely large, and therefore so will the ratio for each species. This simply means that abrasion is no longer an age-limiting factor in these animals.

Comparisons can be made even between unrelated taxa, but care should be taken that (1) foods of a similar abrasiveness are eaten or a correction is made for this, (2) the linear measurements or measurement used are in each case representative of the size of the animals, and not of a specialization peculiar to one of them, and (3) the entire cheek tooth series is considered, not a single tooth, unless a tooth is present that is equally representative of the total attrition for each group. Different teeth may regularly wear at quite different rates in the same individual, and this pattern of differences is somewhat variable between different mammalian groups.

It is perhaps appropriate here to comment on an otherwise valuable paper by White (1959), which rejects explanations of hypsodonty related to adaptation to the external environment. Much comparative information is summarized in this paper. He seems, perhaps intentionally, to ignore the implications of the fact that natural selection is simply the greater reproductive success of those individuals that have a certain genetic constitution. This difference may be small at a given time, but is eventually appreciable with continual variation and recurrent mutation and recombination. White's ontogenetic explanation is consistent with this phylogenetic explanation, which is discussed in greater detail by Simpson (1953). As mentioned above, the origin and evolution of hypsodonty can be related to (but is not a *necessary* result of) increase in size, change in diet (many temperate grasses do contain small silicious bodies), or increase in maximum life span. In addition, greater activity than predicted by the exponental relation mentioned above may also occasionally be involved as a minor component. These seem adequate to explain individually all cases of hypsodonty; although positive evidence for one or more of them is often lacking, the available evidence is in no case inconsistent with them, as it could easily have been if they were not involved.

I am indebted to Morris Skinner of the Frick Laboratory for conversations on this subject, and to Malcolm McKenna of the American Museum of Natural History for critical reading of the manuscript.

Summary

A functional analysis is given of hypsodonty and the other components of resistance to dental attrition in mammals. An index is proposed to compare two or more populations or individuals as to their relative success in this.

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THE ALPHA AND OMEGA OF CURRENT EVOLUTIONARY THOUGHT

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The consummation of a cataract of Darwinian festivities and commemorations held in the course of 1958 and 1959 in many lands and on all continents (with the possible exception of Antarctica) was reached in the week-long Centennial Celebration at the University of Chicago in November of the latter year. Two volumes [1] of the proceedings are now available, and a third, containing papers on science and religion, the procés-verbaux of five panel discussions and a general index to the whole series, is, at the time of writing, still to come (the set will then be available for $25.00).

It might have been expected that, after the inundation of ceremonies, exhibitions, symposia, colloquia, seminars, lectures, broadcasts, critiques, reviews, appreciations, discussions and evaluations of Darwin, his predecessors, his followers, his work and his influence, the final jubilee examination of *Evolution After Darwin,* could be an anticlimax, especially, since most of those who have something worthwhile to contribute to the subject already had had their say (or usually, says). Indeed, G. G. Simpson, himself one of the most profound and brilliant of the performers on the Darwin circuit, has, in an address delivered at the end of the two years of the miscellaneous observances, expressed surfeit, approaching boredom, with the whole business.

Such expectation, happily, is completely unfounded. The organizers of the Centennial displayed both imagination and ingenuity in the range of matters covered and in the selection of contributors. The result is a prodigious compendium and a distinguished exhibit of the alpha and omega (the tangible whole of science rather than the occult one of Teilhard de Chardin) of evolutionary thinking prevalent in the middle of the twentieth century. The celebrants at the Darwinian bicentenary will, no doubt, find many of the opinions now expressed no longer tenable, much of the present emphasis misplaced in the light of their superior knowledge, some of the current evidence misinterpreted. They may smile at some visionary pronouncements, they may be amused by what, no doubt, will seem to them to be oversimplifications. They will search and find in these volumes (either with agreeable surprise or with raised eyebrows, depending on what they know by then) grains of dissent from the general paeans in praise of the prevailing notions about the mechanisms of both organic and cultural evolution. No matter what, they will not fail to be impressed by the amount of factual and conceptual advance the several generations (and especially, the more recent ones) of post-Darwin students have made in the journey towards understanding man and his environment.

For biology, this has been a proud century. The synthetic theory of evolution (for which sentiment still finds *Neo-Darwinian* to be an alluring epithet) emerged towards the end of it as a lofty concept, revealing design in the history of the organic world, yet without yielding to the temptation of seeing purpose in it. And many, if not most, of the living architects of the synthetic theory are represented in the pages of these proceedings (though such names as Fisher, Haldane and Schmalhausen are conspicuous by their absence), making the record of the Chicago commemoration a monument, not so much to Darwin himself, as to the current efforts in genetics, paleontology, biochemistry, ecology, anthropology and all the other disciplines which contributed to the synthesis.

The forty-two articles, authoritative though they may be, are, of course, not of uniform quality. They range through various degrees of originality and inspiration. None of the essays is pedestrian, and many rise to high levels of excitement and nobility. They cover the vast territory from pre-life, through the organic world and its history to man, society and culture. To comment fully on each of the articles would, by far, transcend the space available here; to single out only some for mention would be invidious. Hence merely a listing of the topics considered in the first volume, and even a less complete inventory of the second, will be given here, albeit one-sentence summaries of the exceptionless thoughtful essays are bound to distort their messages and underrate their significance. Before presenting this catalogue, it is a pleasure to record that the planners of the contents of the first volume, in addition to Sol Tax, the editor, were Alfred E. Emerson, Everett C. Olson and the late Karl P. Schmidt, all three, current or past officers of the Society for the Study of Evolution. Assisting Tax in the selection of contributors to the second volume were Ilza

[1] Tax, Sol (ed.). 1960. Evolution After Darwin. vol. 1. The Evolution of Life: Its Origin, History and Future. VIII + 629 pp. $10.00. vol. 2. The Evolution of Man: Mind, Culture and Society. VIII + 473 pp. $10.00. University of Chicago Press, Chicago.

Veith, Sherwood L. Washburn and the late Robert Redfield.

The first volume contains twenty essays. Julian Huxley leads off with a historical introduction to Darwinism. His article is followed by Shapley's discussion of inorganic evolution, cosmology, including the basic hypotheses regarding the origin of the universe, and the increasingly piquant problem of life on other worlds. Gaffron next, in a paper combining the biochemical and philosophical approaches, also deals with this issue, more specifically addressing himself to the question of origin of life, and to the gaps in the logical and evidential contents of scientific knowledge. Evans continues with a summary of the known and unknown in the evolutionary history of viruses.

Two sweeping, but skillfully condensed, views of the whole grand panorama of organic evolution appear next. Rensch, devotes a major share of his essay to a listing of both general and special rules of evolutionary change, to the astonishing number of thirty-three. Simpson, provides, what probably will to most general evolutionists be the pivotal article in the volume, centered on the fossil record, but dealing in a forceful way with many other, including, again the philosophical, aspects of evolution. Ford confines himself largely to the description of the experimental evolutionary studies of the Oxford group. Stebbins attempts to encompass the whole range of living organisms in a consideration of the origin and development of their genetic systems, while Axelrod contributes a monographic treatment of the evolution of flowering plants. Emerson then examines the evolutionary process on the population level, involving the inception and maintenance of group, rather than individual, adaptive properties. Mayr presents a penetrating analysis, leading to a concrete hypothesis, dealing with the long-vexing problem of perfection of structures that may not be advantageous until they are phylogenetically fully developed.

Three articles by geneticists follow. Waddington, manifesting a trenchantly revisionist attitude towards the synthetic theory, calls for a broadening of our outlook on the nature of evolutionary mechanisms. Dobzhansky, in his usual lucid style, illustrates the operation of the various evolutionary forces as revealed by experimental studies in population genetics. Wright, in the only predominantly mathematical work in the volume, summarizes his classical development of quantitative evolutionary theory.

Nicholson's contribution, in the course of a review of the role of population dynamics in evolution, contrasts Darwin's and Wallace's approaches to natural selection. Olson, though ostensibly concerned with morphological and paleontological considerations, in fact, presents a much more general scrutiny of the gaps and deficiencies in the synthetic theory. Contrary to the case in many such critiques, his reservations proceed from a position of understanding the theory, as is also true of the comments by Bates in the following essay on ecology and evolution. Prosser then offers a capsule course on physiological evolution, while Tinbergen analyzes the use of behavioral traits in animal systematics. Gause, the only Russian represented, brings the first volume to a conclusion with a consideration of neoplastic growth, its equivalents in lower organisms, and the problems of cancer chemotherapy. The link to Darwinism is made by framing his essay in a recollection of Metchnikoff's Cambridge commemorative speech at the half-way point in the Darwin century.

The second volume, generally speaking, will have less impact on the readership of this journal. Many of the twenty-two articles in it deal with cultural rather than organic evolution, and, hence, are more oriented on the Lamarckian (which may be a suitable recognition of Lamarck's own sesquicentennial) rather than on the Mendelian approach. This is, indeed, appropriate to aspects of evolution (in the broadest sense of the word) in which mechanisms for transmission of acquired characters are clearly discernible. But, just as Olson sounded a warning in the first volume, so are there voices (notably, that of Julian Steward) in the second, raised in favor of withholding complete commitment to the adequacy of present concepts of cultural evolution.

In general, without implying that the articles by cultural anthropologists and archeologists (Kroeber, Piggott, Bordes, Willey, Braidwood, Adams) deal with matters of indifference to students of organic evolution, those written by anatomists, physiologists, psychologists (Magoun, von Muralt, Gantt, Hilgard, Brosin), and, to a greater degree, those straddling or overlapping several such fields (White, Gerard, Critchley, Hallowell) have a richer biological content. Of even more direct concern to organic evolutionists are Emiliani's account of his methods and unorthodox (used here by no means in a pejorative sense) conclusions on evolutionary chronology, Leakey's first-hand description of recent discoveries of African hominid remains, the comprehensive account by Washburn and Howell of the later stages in the evolution of man, and, finally, Anderson's summarization of information on the domestication of plants. The penultimate article by Muller, setting out some of the considerations involved in the possibility of consciously directing human evolution, is both prophetic and cautionary in intent. The volume closes with a Malthusian peroration by Charles Galton Darwin, the grandson of the Centennial's protagonist.

This brief listing of the contents of 1101

pages obviously accomplishes little more than can jacket blurbs. But, I hope, it will induce its perusers to make direct acquaintance with the volumes under review. Two final comments might be made. Firstly, the twenty-five dollar price tag on the set of three volumes, reasonable though it may be in terms of current publication costs, will deter many who should own it from buying it. Perhaps, after institutional sales have amortized the initial outlay, a popular paperbound edition could be produced. And, second, to end on a pensive note, this is the first collection of its size of papers in biology that I have seen in many a moon, without even a single article containing the term DNA in the title.

BOOKS RECEIVED

Notices will be given under this heading of books of evolutionary interest sent to the Editors. Appearance of a title in this section will not preclude a fuller review in a later issue of the journal.

Termier, H. and G. Termier. 1960. Paléontologie Stratigraphique. 515 pp. 3425 figs. 148 New Frs. Masson. Paris. An illustrated catalogue of fossil types intended as a reference and textbook.

Termier, H. and G. Termier. 1960. Atlas de Paléogéographie. 100 pp. 8 figs. 36 colored maps. 16 New Frs. Masson. Paris. An atlas to supplement the volume noted above.

Jarvik, E. 1960. Théories de l'Évolution des Vertébrés. 104 pp. 30 figs. 20 New Frs. Masson. Paris. A French translation of a Swedish work (lacking a bibliography) on vertebrate paleontology.

Harper Torchbooks. The Science Library. 1960. Paperback reprints by Harper, New York, of monographs, uniformly priced at $1.35. The dates given in parentheses are of the original publications:

Bellairs, Angus d'A. (1957). Reptiles: Life History, Evolution and Structure. 192 pp. 12 figs.

von Bertalanffy, Ludwig. (1952). Problems of Life. VIII + 216 pp.

Cain, A. J. (1954). Animal Species and Their Evolution. 190 pp. 5 figs.

Fuller, John L. and W. Robert Thompson. 1960. Behavior Genetics. IX + 396 pp. illus. $8.95. John Wiley. N. Y. The first textbook specifically devoted to the inheritance of behavioral traits.

Archives de Philosophie. 1960. Vol. 23 (1). L'Evolution et ses Mecanismes, Darwinisme, Polygenisme, Evolution et Philosophie. 168 pp. 7.5 New Frs. A Darwinian symposium containing six essays on evolution emphasizing the philosophical aspects of the subject.

Stokes, William Lee. 1960. Essentials of Earth History. X + 502 pp. illus. $8.75. Prentice-Hall. Englewood Cliffs, N. J. A lavishly illustrated textbook of historical geology for liberal arts students.

Stern, Curt. 1960. Principles of Human Genetics. IX + 753 pp. 265 figs. $9.50. Freeman. San Francisco. The second edition, extensively rewritten and enlarged, of what is generally recognized as the classic textbook of the subject.

Allard, R. W. 1960. Principles of Plant Breeding. XI + 485 pp. illus. $9.00. John Wiley. A textbook of plant breeding orientated on population, biometrical and radiation genetics as well as cytogenetics. Both theoretical and practical aspects are considered.

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The American Institute of Biological Sciences wishes to draw attention to its program of translating and publishing Russian research journals in biology. Currently, these include three sections of the proceedings of the Soviet Academy of Sciences (Biological Sciences, Botanical Sciences, Biochemistry), Plant Physiology, Microbiology, Soviet Soil Science, and the Entomological Review. A number of translations of Russian monographs are also being published. Information about this program may be obtained from AIBS, 2000 P Street, N. W., Washington 6, D. C.

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Vol. XIV *March 1960* No. 1

CONTENTS

Courtship and Sensory Preferences in Inbred Lines of *Drosophila melanogaster*. H. F. HOENIGSBERG and S. KOREF SANTIBANEZ 1
Osteometric Variation and Function in Bats. ROBERT S. BADER and JOHN S. HALL 8
A Study of Plant Competition in Relation to Development. JOSHUA A. LEE 18
Studies on the Japanese Honeybee, *Apis cerana cerana* Fabricius. VIII. Two Opposing Adaptations in the Post-stinging Behavior of Honeybees. SHÔICHI F. SAKAGAMI and YUKIO AKAHIRA 29
Food Preferences of Larval and Adult Drosophila. DONALD M. COOPER ... 41
The Behavior of Cytoplasmic Pollen Sterility in Populations. G. S. WATSON and ERNST CASPARI 56
Latitudinal Variations in Organic Diversity. ALFRED G. FISCHER 64
Stage of Speciation of Two Allopatric Populations of Chorus Frogs (*Pseudacris*). W. FRANK BLAIR and MURRAY J. LITTLEJOHN 82
A Cytogenetic Study of *Clarkia exilis*. FRANK C. VASEK 88
Evolution of Gametophytic and Sporophytic Systems of Self-incompatibility in Angiosperms. KAMLA KANT PANDEY 98
Interaction Between Inversion Polymorphisms of Two Chromosome Pairs in the Grasshopper, *Moraba scurra*. R. C. LEWONTIN and M. J. D. WHITE 116
Notes and Comments
A Critique of "Morphological Integration." WALTER J. BOCK 130
Morphological Integration: a Discussion. ROBERT L. MILLER and EVERETT C. OLSON 132
Determination of Drosophila Karyotypes from Adult Males. FRANCES E. CLAYTON 134
Supernumerary Chromosomes in *Haplopappus gracilis*. R. C. JACKSON 135
Natural Selection in Middle Island Water Snakes (*Natrix sipedon* L.). PAUL R. EHRLICH and JOSEPH H. CAMIN 136
Possible Evidence of Rapid Evolution in Hawaiian Moths. ELWOOD C. ZIMMERMAN 137
Origins of Angiospermous Plants. G. LEDYARD STEBBINS 138
Evolutionary Morphology of Plants. VERNE GRANT 139
Molecular Biology. G. COHEN-BAZIRE 141
Books Received 141
Report of the Secretary 142

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Vol. XIV *June 1960* No. 2

CONTENTS

Mate Preference in Males of Four Species of Gambusiine Fishes. CLARK HUBBS and EXALTON A. DELCO, JR. 145

The Pattern of Interspecific Variation in the Genus *Oryza:* Its Quantitative Representation by Statistical Methods. HIROKO MORISHIMA and HIKO-ICHI OKA 153

Chromosomal Polymorphism in Egyptian Populations of *Drosophila melanogaster.* A. M. MOURAD and G. S. MALLAH 166

The Ecological Significance of Correlation Pleiades. R. L. BERG 171

Evolutionary Consequences of Hybrid Sterility and Vigor in Toads. E. PETER VOLPE 181

The Chorda Tympani and Middle Ear as Guides to Origin and Divergence of Reptiles. NICHOLAS HOTTON III 194

The Genetics of Hybrid Sterility in *Drosophila paulistorum.* LEE EHRMAN 212

Evolution and Variation in Trillium. VI. ICHIRO FUKUDA, YOICHIRO HIRAIZUMI, TAKASHI NARISE and MASATAKA KURABAYASHI 224

Evolution and Variation in Trillium. VII. YOSHIMICHI KOZUKA and MASATAKA KURABAYASHI 232

The Breeding Group and Seed Storage: a Study in Population Dynamics. CARL EPLING, HARLAN LEWIS and FRANCIS M. BALL 238

The Cytoplasmic "Sex-Ratio" Condition in *Drosophila.* G. S. WATSON ... 256

Notes and Comments

Mutant Genes and Linkage Relationships in *Drosophila paulistorum.* CHANA MALOGOLOWKIN and LEE EHRMAN 266

Evolutionary Fundamentals of Plant Breeding. ÁSKELL LÖVE 270

Darwin, and after Darwin. H. G. BAKER 272

On the Possibly Polyphyletic Origin of Reptiles. PETER PAUL VAUGHN 274

Books Received 276

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SEP 16 1960

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INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

Vol. XIV *September 1960* No. 3

CONTENTS

Regional Melanism in Aquatic Beetles. FRANK N. YOUNG 277

Cytogenetics of the Grasshopper *Moraba scurra*. V. Biometric Effects of Chromosomal Inversions. M. J. D. WHITE and LESLEY E. ANDREW ... 284

Genetic Variability in Korean Populations of *Drosophila melanogaster*. YONG KYUN PAIK 298

Therapsids as Mammals. LEIGH VAN VALEN 304

Polyphyletic or Monophyletic Ancestry of Mammals, or: What Is a Class? CHARLES A. REED 314

The Endemic Fish Fauna of Lake Lanao, and the Evolution of Higher Taxonomic Categories. GEORGE SPRAGUE MYERS 323

Allochronic Speciation in Field Crickets, and a New Species, *Acheta veletis*. RICHARD D. ALEXANDER and ROBERT S. BIGELOW 334

Pattern of Chromosome Variability in *Drosophila pseudoobscura*. ERIC TERZAGHI and DANIEL KNAPP 347

Heritability of Geographic Variation in Trunk Segmentation in the Red-Backed Salamander, *Plethodon cinereus*. RICHARD HIGHTON 351

The Evolution of Thermiphilous Water Mites. RODGER MITCHELL 361

Reproductive Isolation Between *Drosophila tolteca* and Related Species. STEWART E. ENSIGN 378

Notes and Comments

Evolution of Communication in Bees and Its Role in Speciation. WARWICK E. KERR 386

Diagnosis of the Classes Reptilia and Mammalia. GEORGE GAYLORD SIMPSON 388

The Antecedents of Man. EDWIN H. COLBERT 392

Fish Evolution in Lake Nyasa. GEORGE SPRAGUE MYERS 394

Evolution of Fishes in Lake Nyasa. GEOFFREY FRYER 396

Genetics and Darwinism. SPENCER W. BROWN 400

Books Received 401

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CONTENTS

Ecological Aspects of Flower Evolution. I. Phyletic Evolution. L. VAN DER PIJL 403

Evolution of Cellular Antigens in Columbidae. J. H. STIMPFLING and M. R. IRWIN 417

Differential Radiosensitivity Among the Probable Genome Donors of Bread Wheat. R. A. PAI and M. S. SWAMINATHAN 427

Meiotic Drive in Natural Populations of *Drosophila melanogaster*. III. Populational Implications of the Segregation-Distorter Locus. YUICHIRO HIRAIZUMI, L. SANDLER and JAMES F. CROW 433

Introgressive Hybridization Between Two Southeastern Treefrogs. JOHN S. MECHAM 445

The Evolutionary Dynamics of Complex Polymorphisms. R. C. LEWONTIN and KEN-ICHI KOJIMA 458

Pseudogamy as an Evolutionary Factor in the Poeciliid Fish *Mollienisia formosa*. CARYL P. HASKINS, EDNA F. HASKINS and RICHARD E. HEWITT .. 473

Isolating Mechanisms in Three Sympatric Treefrogs in the Canal Zone. M. J. FOUQUETTE, JR. 484

Evolutionary Aspects of Aberrant Meiosis in Some Pentatominae (Heteroptera). FRANZ SCHRADER 498

Evolution and Cybernetics. I. I. SCHMALHAUSEN 509

Notes and Comments

Mating Preferences in *Drosophila*. DAVID J. MERRELL 525

Sexual Behavior: a Discussion. H. F. HOENIGSBERG 527

Comments on the Origin of the Amniote Eggs. J. A. TIHEN 528

A Functional Index of Hypsodonty. LEIGH VAN VALEN 531

The Alpha and Omega of Current Evolutionary Thought. I. MICHAEL LERNER 533

Books Received 535

Announcement 535

Index for Volume XIV

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